ANTI-INFLAMMATORY, ANTIPYRETIC AND ANTIBACTERIAL STUDY OF KABASURA KUDINEER CHOORNAM

A Dissertation Submitted to

THE TAMIL NADU Dr. M. G. R. MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfillment of the requirement for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

OCTOBER-2017

DEPARTMENT OF PHARMACOLOGY

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KOVAI ESTATE, KALAPPATTI ROAD,

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Reg. No. 261525808

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CERTIFICATE

This is to certify that the dissertation work entitled **"Anti-inflammatory, antipyretic and antibacterial study of Kabasura kudineer choornam"** was carried out by **Reg. No. 261525808**. The work mentioned in the dissertation was carried out at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamilnadu, for the partial fulfillment for the degree of Master of Pharmacy during the academic year 2016-2017 and is forwarded to the Tamil Nadu Dr. M. G. R. Medical University, Chennai.

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CERTIFICATE

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This research work either in part or full does not constitute any of any thesis / dissertation.

Date:

Place: Coimbatore **Signature of the guide**

DECLARATION

I do here by declare that to the best of my knowledge and belief ,the dissertation work entitled **"Anti-inflammatory, antipyretic and antibacterial study of Kabasura kudineer choornam"** submitted to the Tamil Nadu Dr. M.G.R. Medical university , Chennai, in the partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology,** was carried out at Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, during the academic year 2016-2017.

Date:

Place: Coimbatore **Reg. No: 261525808**

EVALUATION CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"Anti-inflammatory, antipyretic and antibacterial study of Kabasura kudineer choornam"** submitted by **Reg No: 261525808** to the Tamil Nadu Dr. M.G.R. Medical university, Chennai, in the partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work carried out by the candidate during the academic year 2016-2017 at KMCH College of Pharmacy, Coimbatore, Tamil Nadu and the same was evaluated by us.

Examination Center: KMCH College of Pharmacy, Coimbatore

Date:

Place: Coimbatore

Internal Examiner **External Examiner**

Convener of Examination

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Declaration

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DEDICATED TO ALMIGHTY,

MY BELOVED PARENTS,

BROTHERS AND FRIENDS

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"WE ALL CAN WORK; BUT TOGETHER WE WIN"

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Reg No: 261525808

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ABBREVIATIONS

SL.NO	ABBREVIATIONS	FULL FORM
1.	5-HETE	5-Hydroxyeicosatetraenoic acid
2.	ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
3.	AEKKC	Aqueous Extract of Kabasura Kudineer Choornam
4.	ANOVA	Analysis of Variance
5.	ATP	Adenosine Triphosphate
6.	CD4	Cluster of Differentiation 4
7.	COX	Cyclooxygenase
8.	DMSO	Dimethyl Sulfoxide
9.	DPPH	1,1 Diphenyl Picryl Hydrazine
10.	FRAP	Ferric Reducing Anti-oxidant Power
11.	GC-MS	Gas Chromatography–Mass Spectrometry
12.	HPTLC	High Performance Thin Layer Chromatography
13.	ICAM1	Intercellular Adhesion Molecule 1
14.	IFN	Interferon
15.	Ig	Immunoglobulins
16.	IL	Interleukin
17.	LOX	Lipoxygenase
18.	LPS	Lipopolysaccharides
19.	LT	Leukotrienes
20.	MIC	Minimum Inhibitory Concentration
21.	NK cells	Natural Killer Cells
22.	NSAID	Non-Steroidal Anti-Inflammatory Drugs
23.	OECD	Organisation for Economic Co-operation and Development
24.	OTC	Over-The-Counter

25.	PAR1	Protease-Activated Receptor 1
26.	PGs	Prostaglandins
27.	PMN	Polymorphonuclear Neutrophils
28.	ROS	Reactive Oxygen Species
29.	SEM	Standard Error Mean
30.	TNF-α	Tumour Necrosis Factor-α
31.	VCAM1	Vascular Cell Adhesion Molecule 1

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1. INTRODUCTION

HERBAL MEDICINES

The term “medicinal plant” include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. The word “herb” has been derived from the Latin word, “herba” and an old French word “herbe”. [1]

We have so much benefit from plants in their most natural state. The famous father of medicine, Hippocrates was quoted as saying: Let thy food be thy medicine, and thy medicine shall be thy food. We should be so grateful to plants for the food, health, and healing remedies that they have provided, and continue to provide us with. They have been used as medicine and as diverse healing modalities for anything from external to internal infections, mental and emotional imbalances, as well as for every physical illness. [2]

Herbal medicines were used in ancient Chinese, Greek, Egyptian and Indian medicine for various therapeutic purposes. The history of herbal medicines is as old as human civilization. The knowledge of herbal medicines has been transferred from generation to generation and this is the root of allopathic medicine and its derivatives. According to World health organisation, it is estimated that 80% of the world’s population still depend mainly on traditional medicines for their health care.

The traditional knowledge and experiences supports the discovery of variety of natural and semisynthetic compounds. Nowadays, the usage of medicinal plants has increased day by day not only for primary healthcare but also to treat chronic diseases like cancer, diabetes mellitus, liver disorders, rheumatic pains etc.

Thus it is a well-known fact that the traditional systems of medicines has always played important role in meeting the global health care needs. They are continuing to do so at present and shall play major role in future as well. The medicinal system which are considered to be Indian in origin or the systems of medicine, which have come to India from outside, got assimilated in to Indian culture are known as Indian systems of

medicine. India has the unique distinction of having six medicinal systems of in this category. They are Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy. Though Homoeopathy came to India in 18th Century, it completely assimilated in to the Indian culture and got enriched like any other traditional system hence it is considered as part of Indian Systems of Medicine. Apart from these systems there are large number of healers in the folklore stream who have not been organized under any category. [3]

Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases increased the use of plant materials as medicines for a wide variety of human diseases. [1]

Pain and fever are being the most common complaints associated with inflammation. The NSAIDs used in the inflammatory conditions do not cure or remove the underlying cause of the disease but they only modify the inflammatory response to the disease. Large numbers of NSAIDs are available in the market with their advantages and disadvantages. Though there are effective drugs like aspirin, indomethacin, phenylbutazone, etc., these drugs are not entirely free of side effects and have their own limitation. Thus there is a need to develop newer and safer drugs. NSAIDs use is frequently limited by gastrointestinal side effects, ranging from dyspepsia to life threatening bleeding from ulceration. It is believed that NSAIDs by inhibiting COX pathway causes inhibition of prostaglandins synthesis, which are responsible for maintaining gastric mucosal integrity.

Pathogenic bacteria have always been thought to be a considerable cause of morbidity and mortality in humans. Although different pharmaceutical companies have introduced a number of new anti-bacterials in the last years, but resistance to these agents has also increased and has now become a worldwide problem Herbal medicines used in ayurveda remain the major source of health care for the world's population. World health organisation has recognized herbal medicine as an important building block for primary health care of vast countries like India. [4]

SIDDHA MEDICINE

Siddha medicine is the traditional medical system that is widely practised in South India. It was enriched with ethnic medical knowledge of Tamil people, native of rich biodiversity zone of Western Ghats. [5]

It is believed to be originated more than 10,000 years ago. Siddha system consider human and nature as a part of closed system. Palm leaf manuscripts says that the Siddha system was first described by Lord Shiva to his wife Parvathy. Parvathy explained all this knowledge to her son Lord Muruga. He explained all these knowledge to his disciple sage Agasthiyar. Agasthiyar taught the knowledge about siddha to 17 Siddhars and they spread it to the human beings.

The word Siddha came from the word 'siddhi'. The persons who attained this are called Siddhars. They wrote literatures in classical Tamil language for all branches of science and it is mainly practiced in southern part of India. [6]

FUNDAMENTAL PRINCIPLES OF SIDDHA MEDICINE

The universe consists of two essential entities, i.e. matter and energy. The Siddha medicine called them Siva and Shakti. Matter cannot exist without energy inherent in it and vice versa. The two co-exist and are inseparable. Their names are Munn (solid), Neer (fluid), Thee (radiance), Vayu (gas) and Aakasam (ether). These five elements (Bhutas) are present in every substance, but in different proportions. Earth, water, fire, air and ether are symbols of these 5 elements.

The human being is made up of these five elements, in different combinations. The physiological function in the body is mediated by three substances (dravayas), which are made up of these five elements. They are Vatham, Pitham, and Karpam. In each and every cell of the body these three doshas i.e. vatham, pitham, karpam co-exist and function harmoniously. The tissues are called dhatus. Akasam and Vayu forms Vatham and it controls the nervous actions such as movement, sensation, etc. Pitham is formed by Thee and controls the metabolic activity of the body, digestion, assimilation, warmth, etc. Kapam is formed by Munn and Neer and controls stability. When their equilibrium upsets diseases sets in. [7]

Treatment is aimed at restoring balance to the mind-body system. Diet and lifestyle plays a major role not only in maintaining health but also in curing diseases. This concept of the Siddha medicine is known as pathiam and apathiam, which is essentially a list of do's and don'ts. [8]

Generally the basic concepts of the siddha medicine and the ayurveda medicine are similar. The important difference is that the siddha medicine identifies the predominance of vatham, pitham and karpam in childhood, adulthood and old age. But in ayurvedic medicine, it is totally reversed that is, it identifies the dominance of karpam, vatham, pitham in childhood, old age and adults. [9]

KABASURA KUDINEER CHOORNAM

It is a compound formulation consisting of fifteen herbal ingredients. It is commonly used for the treatment of fever with or without respiratory infection. It was prescribed in large during the epidemic of Swine flu as a prophylactic and media reports gave a renaissance to this official Siddha formulation. Siddha medicinal preparations have been classified as 32 internal and 32 external medicinal forms and choornam is one among the internal medicinal form. The current drug is further classified as kudineer choornam which means a drug to be made into decoction and consumed. [10]

Kudineer

Kudineer is the name given to the Siddha formulation in which the whole plant (plants) or the particular part of plant (plants) are grinded into coarse powder. The obtained preparation is called ‘choornam’. It is then made into kudineer by adding water and heated till water reduces to 1/4th or 1/8th of its volume. It is then filtered and filtrate is used. Dose of the kudineer is generally 30 ml before food, three to four times a day. Lifetime of prepared kudineer is 1 Samam (3 hours). The method of preparation of kudineer is simple and the phytoconstituents do not undergo any major change while processing and preparation, unlike other traditional formulations. [11]

INFLAMMATION

Inflammation is the word obtained from the Latin word *inflammo*. It is a part of the complex biological response of body tissues to various harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective response involving immune cells, blood vessels, and molecular mediators. The important function of inflammation is to eliminate the initial cause of cell injury, clear out the necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. [12]

CLASSICAL SIGNS OF INFLAMMATION

Inflammation has been studied for thousands of years and Celsus in 30 A.D. described the four classical signs of inflammation [13]

- Redness (rubor)

An acutely inflamed tissue appears red, due to dilatation of small blood vessels within the damaged area (hyperemia).

- Swelling (tumor)

Swelling which occurs from edema is due to the accumulation of fluid in the extravascular space as part of the inflammatory fluid exudate, and to a much lesser extent, from the physical mass of the inflammatory cells migrating into the affected area.

- Heat (calor)

Increase in temperature is due to increased blood flow (hyperemia) through the region, resulting in vascular dilation and the delivery of warm blood to the area.

- Pain (dolor)

Pain results from the stretching partly and distortion of tissues due to inflammatory edema and, in part from some of the chemical mediators of acute inflammation, especially bradykinin and some prostaglandins.

- Loss of function (functiolaesa)

Loss of function, a well-known consequence, which was added by Virchow (1821-1902) to the list of features described in Celsus written work. Loss of function may occur from severe swelling that prevents movement in the area or from the pain that inhibits mobility. [14]

CAUSES OF INFLAMMATION

- Microbial infections**:** Microbes including viruses, bacteria, protozoa, fungi and various parasites.
- Hypersensitivity reactions
- Physical agents, irritant and corrosive chemicals: Physical trauma, ultraviolet or other ionizing radiation, burns or excessive cooling ('frostbite') may cause tissue damage leading to inflammation. Corrosive chemicals such as acids, alkalis, oxidizing agents are also inflammatory stimulus that can cause direct tissue damage.
- Tissue necrosis: Lack of oxygen or nutrients results into inadequate blood flow and it is a potent inflammatory stimulus that can cause the death of tissues. [15][12]

ROLE OF INFLAMMATION

1. Physiological role of inflammation

   It can eliminate the cause of inflammation and thus helps to minimize tissue damage. It prevents the spreading of the cause of inflammation. Inflammation also helps to activate processes of regeneration and repair. Without inflammation, the tissues are not capable of healing.

2. Pathological role

   It involves excessive or long-lasting reaction which leads to tissue damage. And also have role in pathogenesis of many diseases. [16]

SYSTEMIC EFFECTS OF INFLAMMATION

Both acute and chronic inflammation, even if well localized, can have effects on the whole body. The main ones are:

- Leukocytosis

  Leukocytosis is a common feature and it is condition in which there is an abnormal increase in number of circulating white blood cells. A general rule is that increased neutrophils indicate a bacterial infection whereas increased lymphocytes are most likely to occur in viral infections.

- Fever

  Fever is most often associated with inflammation that has an infectious cause, although there are some non-infectious febrile diseases. The elevation of body temperature is thought to improve the efficiency of leukocyte killing and may also impair the replication of many invading organisms.

- Endotoxemia

  Sepsis is the term used for disease due to toxic bacterial products circulating in the blood. Endotoxemia specifically refers to circulating gram-negative bacterial toxic products. There are some cell wall products released from gram-positive bacteria that can have a similar toxic effect. [15]

TYPES OF INFLAMMATION

By considering the defence capacity of the host and duration of response, inflammation can classified as;

- Acute inflammation
- Chronic inflammation

Acute inflammation is of short duration, enduring less than 2 weeks and represents the early body reaction, resolves quickly and is usually followed by healing.

The main features of acute inflammation are:

- Accumulation of fluid and plasma at the affected site
- Intravascular activation of platelets

- Polymorphonuclear neutrophils as inflammatory cells

Chronic inflammation is of longer duration. The important feature of chronic inflammation is the presence of chronic inflammatory cells such as lymphocytes, plasma cells, macrophages, granulation tissue formation, and in specific situations as granulomatous inflammation. Chronic inflammation occurs either after the causative agent of acute inflammation persists for a long period of time, or the stimulus is such that it induces chronic inflammation from the beginning.

ACUTE INFLAMMATION

It can be divided into following two events:

- Vascular events
- Cellular events

❖ **VASCULAR EVENTS**

Vascular events comprise the alteration in the microvasculature (arterioles, capillaries and venules) and these alterations include

- Haemodynamic changes
- Changes in vascular permeability

Haemodynamic Changes

- ✓ Transient vasoconstriction of arterioles. The blood flow may be restored in 3-5 seconds while with more severe injury the vasoconstriction may last for about 5 minutes.
- ✓ Persistent progressive vasodilatation is responsible for redness and warmth at the spot of acute inflammation.
- ✓ Progressive vasodilatation, sequentially, may elevate the local hydrostatic pressure resulting in transudation of fluid into the extracellular space, responsible for swelling at the local site of acute inflammation.
- ✓ Slowing or stasis of microcirculation follows which causes increased

concentration of red cells, and thus, raise the viscosity of blood.

- ✓ Stasis is followed by leucocytic margination i.e. peripheral orientation of leucocytes (primarily neutrophils) along the vascular endothelium. The leucocytes attach to the vascular endothelium briefly, and then migrate through the gaps between the endothelial cells into the extravascular space.[17]

Changes in vascular permeability

In acute inflammation, the capillary hydrostatic pressure increases, and there is also escape of plasma proteins into the extravascular space due to increased vascular permeability (endothelial contraction allowing proteins to escape between cells). As a result, much more fluid leaves the vessels than is returned to them. The net escape of protein-rich fluid is called exudation and the protein rich fluid is called an exudate.

The increase in vascular permeability in acute inflammation involves two mechanism

- Chemical mediators of acute inflammation may cause retraction of endothelial cells leaving intercellular gaps (chemical mediated vascular leakage).
- Toxins and physical agents may cause necrosis of vascular endothelium which may lead to an abnormal leakage (injury induced vascular leakage).

In short vascular permeability increases due to endothelial contraction, retraction or injury mediated by leukocytes. [15]

- ❖ **CELLULAR EVENTS**

The cellular phase of inflammation consists of 2 processes:

1) Exudation of leucocytes; and
2) Phagocytosis

Exudation of leucocyte

Various leukocytes, mainly neutrophils, are critically involved in the initiation and maintenance of inflammation. These cells must be able to move to the site of injury from their usual location in the blood, therefore mechanisms exist to recruit and direct leukocytes to the appropriate place. The process of leukocyte movement from the blood

to the tissues through the blood vessels is known as extravasation, and can be broadly divided up into a number of steps:

1. **Leukocyte margination and endothelial adhesion:**

The white blood cells within the vessels which are generally centrally located move peripherally towards the walls of the vessels. Activated macrophages in the tissue release cytokines such as IL-1 and TNF α, which bind to their respective G protein-coupled receptors on the endothelial wall. Signal transduction induces the immediate expression of P-selectin on endothelial cell surfaces. This receptor binds weakly to carbohydrate ligands on the surface of leukocytes and causes them to "roll" along the endothelial surface as bonds are made and broken.

Cytokines from injured cells prompt the expression of E-selectin on endothelial cells, which functions alike to P-selectin. Cytokines also induce the expression of integrin ligands such as ICAM-1 and VCAM-1 on endothelial cells, which facilitate the adhesion and further slow leukocytes down. These weakly bound leukocytes are free to separate if not activated by chemokines produced in injured tissue. Activation increases the affinity of bound integrin receptors for ICAM-1 and VCAM-1 on the endothelial cell surface, firmly binding the leukocytes to the endothelium.

2. **Migration across the endothelium via the process of diapedesis:**

It is known as transmigration. Chemokine gradients stimulate the adhered leukocytes to move between adjacent endothelial cells. Using adhesion molecules eg. ICAM-1 the endothelial cells retract and the leukocytes pass through the basement membrane into the surrounding tissue.

3. **Movement of leukocytes within the tissue via chemotaxis**:

Leukocytes reaching the tissue interstitium bind to extracellular matrix proteins through expressed integrins and CD44 to prevent them from leaving the site. A variety of molecules behave as chemoattractants. For e.g. potent chemotactic substances for neutrophils are leukotriene B_4, cytokines, soluble bacterial products, components of complement system.

Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

i. Polymorphonuclear neutrophils (PMNs), sometimes called microphages which appears early in acute inflammatory response
ii. Circulating monocytes and fixed tissue mononuclear phagocytes, commonly called as macrophages.

Neutrophils and macrophages on reaching the tissue spaces produce several proteolytic enzymes—lysozyme, protease, collagenase, elastase, lipase, proteinase, gelatinase, and acid hydrolases. These enzymes degrade collagen and extracellular matrix. The microbe undergoes the process of phagocytosis by polymorphs and macrophages and involves the following 3 steps:

a) Recognition and attachment

b) Engulfment

c) Killing and degradation

Recognition and attachment

By the expression of surface receptors on macrophages which recognise microorganisms: mannose receptor and scavenger receptor phagocytosis is initiated. When the microorganisms are coated with specific proteins, opsonins, from the serum or they get opsonised the process of phagocytosis is further improved. Opsonins create a bond between the cell membrane of phagocytic cell and bacteria. The major opsonins present in the serum and their matching receptors on the surface of phagocytic cells (PMNs or macrophages) are as under:

- IgG opsonins the Fc fragment of immunoglobulin G; it is the naturally occurring antibody in the serum that coats the bacteria while the PMNs possess receptors for the same.
- C3b opsonins the fragment produce by activation of complement pathway. It is strongly chemotactic for attracting PMNs to bacteria.

- Lectins are carbohydrate-binding proteins in the plasma which bind to bacterial cell wall.

Engulfment

The opsonised particle bound to the surface of phagocyte is equipped to be engulfed. This is accomplished by development of cytoplasmic pseudopods around the particle due to activation of actin filaments under cell wall, enveloping it in a phagocytic vacuole. Eventually, the plasma membrane enclosing the particle breaks from the cell surface so that membrane lined phagocytic vacuole or phagosome lies internalised and free in the cell cytoplasm. The phagosome fuses with one or more lysosomes of the cell and form larger vacuole called phagolysosome.

Killing and degradation

It is the stage of killing and degradation of microorganism to dispose it off justifying the function of phagocytes as scavenger cells. The microorganisms after being killed by antibacterial substances are degraded by hydrolytic enzymes. However, this mechanism fails to kill and degrade some bacteria like tubercle bacilli. [17][[18]

CHEMICAL MEDIATORS OF INFLAMMATION [18]

1. Plasma derived
2. Cell derived

Table 1: Plasma-derived mediators

Name	Produced by	Description
Membrane attack complex	*Complement system*	A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The combination and activation of this range of complement proteins forms the membrane attack complex, which is able to introduce into bacterial cell walls and causes cell lysis resulting bacterial death.

Plasmin	*Fibrinolysis system*	Able to break down fibrin clots. Also plasmin cleaves the complement protein C3, and activate Factor XII.
Thrombin	*Coagulation system*	Thrombin cleaves the soluble plasma protein fibrinogen to produce insoluble fibrin. Fibrin then aggregates to form a blood clot. Through PAR1 receptor thrombin can bind to cells to trigger several other inflammatory responses, such as production of chemokines and nitric oxide.
Factor XII (*Hageman Factor*)	Liver	It is a protein that circulates inactively. But get activated by collagen, platelets, or exposed basement membranes via conformational change. On activation it is able to activate three plasma systems involved in inflammation: the kinin system, fibrinolysis system, and coagulation system.
Bradykinin	Kinin system	Bradykinin cause vasodilation, increase vascular permeability, smooth muscle contraction, and also pain. Bradykinin is a vasoactive protein.
C3	*Complement system*	C3 Cleaves to produce C3a and C3b. C3a can produce the histamine release by mast cells and thereby produce vasodilation. C3b can bind to bacterial cell walls and act as an opsonin as a target for phagocytosis.
C5a	*Complement system*	Stimulates histamine release by mast cells. Histamine can produce vasodilation. Through chemotaxis it is also able to act as a chemoattractant to direct cells to the site of inflammation.

Table 2: Cell-derived mediators

Name	Type	Source	Description
Lysosome granules	*Enzymes*	Granulocytes	Contains a large variety of enzymes that perform a number of functions and are able to break down a number of substances, some of which may be plasma-derived proteins that allow these enzymes to act as inflammatory mediators.
Histamine	*Monoamine*	Mast cells and basophils	Stored in preformed granules, histamine is released in response to a number of stimuli. It causes arteriole dilation, increased venous permeability, and a wide variety of organ-specific effects.
IFN-γ	*Cytokine*	T-cells, NK cells	This interferon called macrophage-activating factor, and is especially important in the maintenance of chronic inflammation. Also have antiviral, immunoregulatory, and anti-tumour properties.
IL-8	*Chemokine*	Primarily macrophages	Activation and chemoattraction of neutrophils. Have weak effect on monocytes and eosinophils.

Leukotriene B_4	*Eicosanoid*	Leukocytes, cancer cells	Have the ability to mediate leukocyte adhesion and activation. And allow them to bind to the endothelium and migrate across it. In neutrophils, it is also a chemoattractant. And it is able to induce the formation of reactive oxygen species, followed by the release of lysosomal enzymes by these cells.
IL-1 and TNF-α	*Cytokines*	Primarily macrophages	IL-1 and TNF-α induce many similar inflammatory reactions such as fever, production of cytokines, endothelial gene regulation, chemotaxis, leukocyte adherence, activation of fibroblasts. Also responsible for the systemic effects of inflammation, such as loss of appetite and increased heart rate. In additionally TNF-α inhibits osteoblast differentiation.
LTC_4, LTD_4	*Eicosanoid*	Eosinophils, mast cells, macrophages	These three Cysteine-containing leukotrienes contract lung airways, increase micro-vascular permeability, stimulate mucus secretion, and promote eosinophil-based inflammation in the lung, skin, nose, eye, and other tissues.

5-oxo-eicosatetraenoic acid	*Eicosanoid*	leukocytes, cancer cells	Potent stimulator of neutrophil chemotaxis, lysosome enzyme release, and ROS formation; monocyte chemotaxis; and with even greater potency eosinophil.
5-HETE	*Eicosanoid*	Leukocytes	Metabolic precursor to 5-oxo-eicosatetraenoic acid, it is a less potent stimulator of neutrophil chemotaxis, lysosome enzyme release, and reactive oxygen species formation; monocyte chemotaxis; and eosinophil chemotaxis, lysosome enzyme release, and reactive oxygen species formation.
Prostaglandins	*Eicosanoid*	Mast cells	A group of lipids that can cause vasodilation, fever, and pain.
Nitric oxide	*Soluble gas*	Macrophage, endothelial cells, some neurons	Potent vasodilator, relaxes smooth muscle, reduces platelet aggregation, aids in leukocyte recruitment, direct antimicrobial activity in high concentrations.

THE INFLAMMATORY CELLS

The cells involved in acute and chronic inflammation are circulating leukocytes, plasma cells and tissue macrophages.

- Polymorphonuclear neutrophils are acute inflammatory cells, which are involved in initial phagocytosis of bacteria and foreign bodies, engulfment of antigen-antibody complexes.
- Monocytes are chronic inflammatory cells which are involved in bacterial phagocytosis and regulates lymphocyte response.
- Lymphocytes are chronic inflammatory cells which are involved in humoral and cell mediated immune responses and regulate macrophage responses.
- Eosinophils are chronic inflammatory cells which are involved during allergic states and parasitic infestations.
- Basophils containing electron dense molecules and functions as receptor for Ig E molecules
- Plasma cells are derived from B cells and it is larger than lymphocytes with more abundant cytoplasm and eccentric nucleus. Their number increased during prolonged infection with immunological responses, hypersensitivity states and multiple myeloma.
- Giant cells exist in normal tissues. In chronic inflammation when macrophages fail to deal with particles to be removed, they fused together and form multinucleated giant cells. Besides, morphologically distinct giant cells appear in some tumours also. [17]

CHRONIC INFLAMMATION

Chronic inflammation, is a host response to an inciting stimulus. Chronic inflammation is characterized by inflammation, tissue destruction, and attempts at repair all happening at once. Inflammation does not have as much rubor (redness) or calor (heat) as in the acute reaction. Also, exudates aren't so grossly apparent as in acute inflammation. Because of the fibroplasia and neovascularization, areas affected

by chronic inflammation tend to be slightly swollen and firm. If fibrosis is extensive the lesions can be large and disfiguring. Fibrosis (granulation tissue) is the best indicator that the inflammatory response is chronic. [15]

Chronic inflammation have prolonged duration. Chronic inflammation in contrast to acute inflammation is characterised by the vascular changes, edema and a predominantly neutrophilic infiltrate. Chronic inflammation is distinguished by

- Infiltration with mononuclear cells which includes macrophages, lymphocytes and plasma cells
- Tissue destruction, largely induced by the products of the inflammatory cells.
- Repairing which involves new vessel proliferation (angiogenesis) and fibrosis.

Acute inflammation may progress to chronic inflammation. This change occurs when the acute response cannot be resolved, either because of the persistence of the inflammatory agent or because of the intervention with the normal process of healing.

Chronic inflammation can be caused by one of the following 3 ways:

- Recurrent attacks of acute inflammation : In recurrent urinary tract infection leading to chronic pyelonephritis
- Chronic inflammation following acute inflammation: It occurs when tissue destruction is extensive, or the bacteria survive and persist in small numbers at the site of acute inflammation chronic inflammation may occurs. E.g. for such situation is osteomyelitis, pneumonia terminating in lung abscess.
- Chronic inflammation starting *de novo:* e.g. infection with Mycobacterium tuberculosis

TYPES OF CHRONIC INFLAMMATION

- **Non-specific** inflammation, occurs when the irritant substances causes an inflammatory reaction with the formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.

- **Specific,** when the injurious agent causes a characteristic histologic tissue response e.g. tuberculosis, leprosy.

According to histopathological changes chronic inflammation can be classified as:

- **Chronic non-specific inflammation:** It is characterised by non-specific inflammatory cell infiltration e.g. chronic osteomyelitis, lung abscess. An alternative of this type is chronic suppurative inflammation. This type of inflammation is characterised by infiltration of polymorphs and abscess formation additionally. e.g. actinomycosis.
- **Chronic granulomatous inflammation:** Important feature is the formation of granulomas e.g. tuberculosis, leprosy, syphilis etc.

GRANULOMATOUS INFLAMMATION

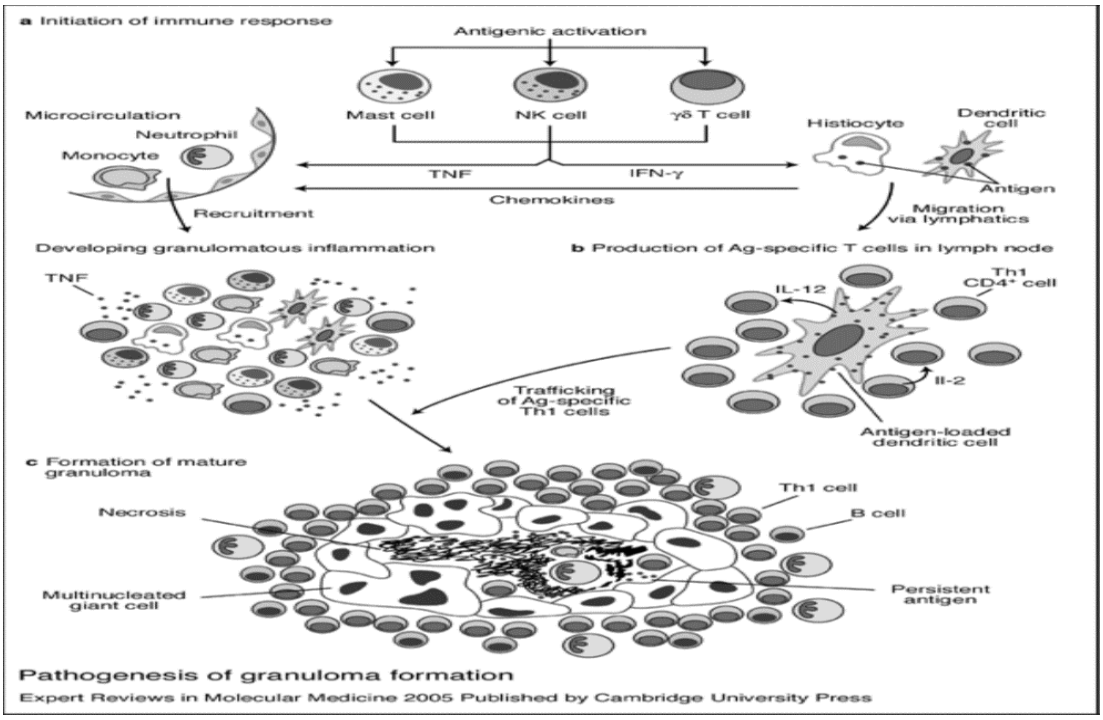
Chronic inflammation characterised by the aggregates of activated macrophages that assume an epithelioid appearance.

The causes of or conditions in which granuloma develops are

- Bacterial infections
- Fungal infections
- Parasites
- Foreign bodies
- Immune conditions [19]

PATHOGENESIS OF GRANULOMA FORMATION

Figure 1: Pathogenesis of granuloma formation



FEVER

Fever is also known as pyrexia or febrile response. It is defined as the elevation of temperature in the preoptic zone of frontal area of hypothalamus above thermal set point of 37 ^{0}C. A fever can be an only symptom in variety of medical conditions and it may be due to viral, bacterial & parasitic infections, systemic conditions, side effects of medication and even cancer. About 30% children and 75% adults visit the healthcare centres for the fever. [20]

RANGE FOR NORMAL TEMPERATURES

A wide range for normal temperatures are found. Central temperatures, such as rectal temperatures, are much more precise than peripheral temperatures. Fever is mostly agreed to be present if the elevated temperature is caused by a raised set point and:

- Temperature in the anus (rectum/rectal) is at or over 37.5–38.3 °C (99.5–100.9 °F)
- Temperature in the mouth (oral) is at or over 37.7 °C (99.9 °F)
- Temperature under the arm (axillary) or in the ear (tympanic) is at or over 37.2 °C (99.0 °F)

Table 3: Temperature classification

Type	Temperature
Hypothermia	< 35.0 ^{0}C (95.0 ^{0}F)
Normal	36.5 – 37.5 ^{0}C (97.7-99.5 ^{0}F)
Fever	>37.5 or 38.3 ^{0}C (99.5 or 100.9 ^{0}F)
Hyperthermia	37.5 or 38.3 ^{0}C (99.5 or 100.9 ^{0}F)
Hyperpyrexia	40.0 or 41.0 ^{0}C (104.0 or 105.8 ^{0}F)

HYPERPYREXIA

It is an extreme elevation of body temperature. Hence it is considered as a medical emergency condition. It may indicate a serious underlying condition or lead to problems including permanent brain damage, or death. The most common cause of hyperpyrexia is an intracranial hemorrhage. Other possible causes include Kawasaki syndrome, sepsis, neuroleptic malignant syndrome, drug overdose, serotonin syndrome, and thyroid storm.

HYPERTHERMIA

It is an example of a high temperature. It is not a fever. It occurs due to several causes including heatstroke, neuroleptic malignant syndrome, malignant hyperthermia, stimulants such as substituted amphetamines and cocaine, idiosyncratic drug reactions, and serotonin syndrome.

Infections are the most important cause of fevers. Infections generally related with hyperpyrexia consist of roseola, measles and enteroviral infections. Immediate aggressive cooling to less than 38.9 °C (102.0 °F) has been found to improve survival. Hyperpyrexia and hyperthermia are different wherein, in hyperpyrexia the body's temperature regulation mechanism sets the body temperature above the normal temperature. Then inorder to attain the normal body temperature, heat is produced while in hyperthermia due to an external source the body temperature rises above its set point.

TYPES OF FEVER

1. Continuous fever: Throughout the day the temperature remains above normal and does not fluctuate more than 1 °C in 24 hours.
2. Intermittent fever: The temperature elevation is present only for a certain period, after that cycling back to normal.
3. Remittent fever: Throughout the day the temperature remains above normal and in 24 hours temperature fluctuates more than 1 °C.
4. Pel-Ebstein fever: A specific kind of fever associated with Hodgkin's lymphoma, being high for one week and low for the next week and so on.[21]

GENESIS OF FEVER

Inflammatory mediators (i.e., cytokines, namely interleukin-1, interleukin-6, tumor necrosis factor, and others) that are predominantly released by activated peripheral mononuclear phagocytes and other immune cells. [22]

PYROGENS

A pyrogen is a substance that induces fever. They are two types. Endogenous and exogenous pyrogens.

- **Endogenous pyrogens**

All endogenous pyrogens are cytokines. Cytokines are the molecules that are a part of the immune system and are produced by activated immune cells. It causes the increase in the thermoregulatory set point in the hypothalamus. Interleukin 1 (α and β) and interleukin 6 (IL-6) are major endogenous pyrogen. While interleukin-8, tumor necrosis factor-β, macrophage inflammatory protein-α and macrophage inflammatory protein-β as well as interferon-α, interferon-β, and interferon-γ are the minor endogenous pyrogens. Tumor necrosis factor-α also acts as a pyrogen. It is mediated by interleukin 1 (IL-1) release.

- **Exogenous pyrogens**

LPS is a cell wall component of gram-negative bacteria. Lipopolysaccharide-binding protein (LBP) is an immunological protein which binds to LPS. The LBP–LPS complex binds to the CD14 receptor of a nearby macrophage. The synthesis and release of various endogenous cytokine factors, such as interleukin 1 (IL-1), interleukin 6 (IL-6), and the tumor necrosis factor-α occurs after the binding to CD14 receptor. Thus the exogenous factors can cause release of endogenous factors, which in turn, initiate the arachidonic acid pathway. Through the inhibition of ATP production by mitochondria, the highly toxic metabolism-boosting supplement 2, 4 -Dinitrophenol induces [hyperthermia] high body temperature. Instead of producing ATP, the energy of the proton gradient is lost as heat. [21]

PATHOPHYSIOLOGY OF FEVER

Fever is recognized as a complex, coordinated, autonomic, behavioral response and neuroendocrine which occurs due to acute phase reaction to immune challenge. [23] Natural defence system of the human body is activated whenever body finds any infectious agent in order to create an unfavourable environment for the survival of infectious agent. The infectious agent or damaged tissues initiate the increase production of proinflammatory mediators cytokines such as interleukin 1β, β, α and TNF-α which enhance the formation of prostaglandin E_2 (PGE_2) near the peptic hypothalamus area and the prostaglandin in turn act on the hypothalamus to elevate the body temperature. [24]

A centre in the hypothalamus controls the balance between heat loss and heat production that regulates normal body temperature. Fever occurs due to this disturbance of hypothalamic 'thermostat', which leads to the set point of body temperature being raised. [25]

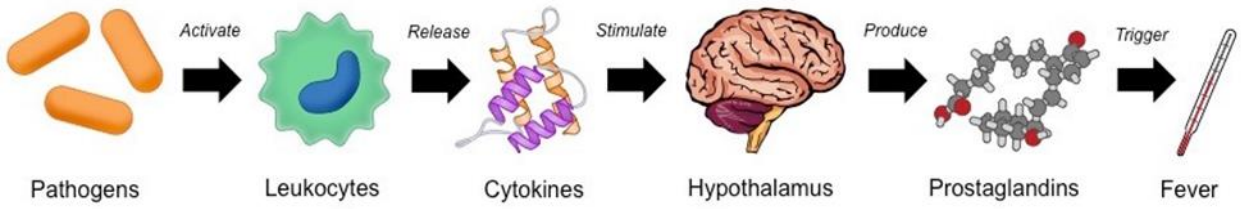


Figure 2: Pathophysiology of fever

So whenever the body temperature becomes high, blood vessels dilated and sweating increases to reduce the high temperature. But when the body temperature is low then vasoconstriction occurs to protect the internal body temperature. Increase temperature as in case of fever leads to faster disease progression due to increased tissue catabolism, dehydration and persisting complaints as in case of HIV infection and other chronic infections. [24]

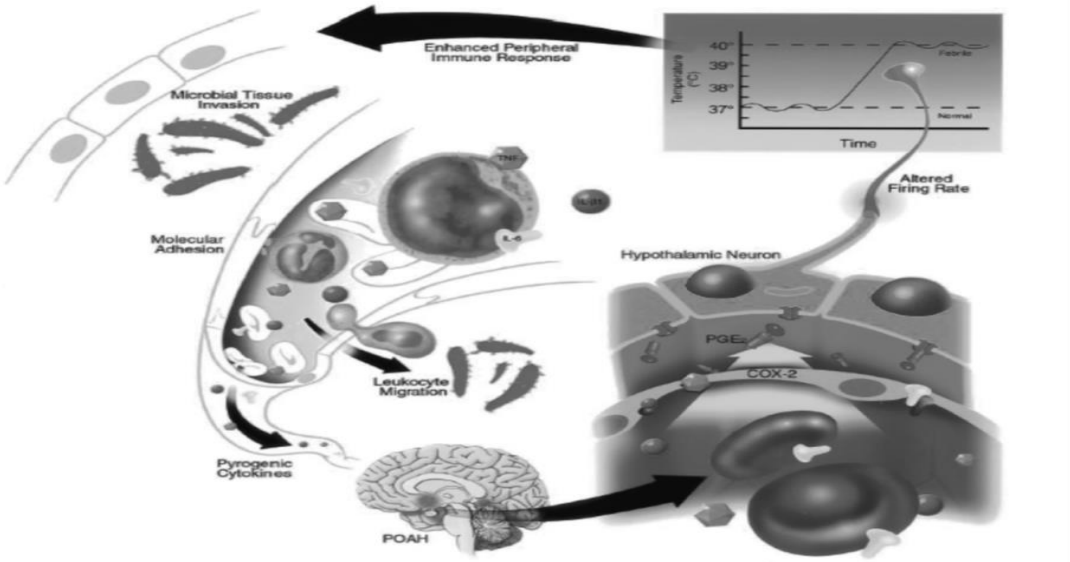
MOLECULAR MECHANISM OF FEVER

Distinct members of Toll receptors in macrophages receptor family recognize different and specific microbial components, but biosynthesis and releases same endogenous pyrogens, such as IL-1β, TNF, and IL-6. [23]

Cytokines which are transported by the bloodstream could act at sites lacking a tight blood–brain barrier, the so-called circumventricular organs. Alternatively, circulating cytokines could interact with their specific receptors on brain endothelial cells or perivascular cells and thereby stimulate these cells to release pyrogenic mediators into the abluminal brain tissue. It has been proposed that fever-promoting cytokines are transported from the blood into the brain via specific carriers. An assumed manifestation of a febrile response produced by these mechanisms is termed as the humoral hypothesis of fever induction. [22]

These pyrogenic cytokines acts on organum vasculosum area of the brain known as laminae terminalis leading to activation of the enzyme cyclo-oxygenase-2 (COX-2) that results in release of prostaglandin E_2 (PGE_2), which binds to receptors in the hypothalamus leading to an increase in heat production and a decrease in heat loss until the temperature in the hypothalamus reaches an elevated set-point. [23]

Figure 3: Molecular mechanism of fever



HOME REMEDIES FOR TREATMENT OF FEVER

When there is mild fever home remedy is sufficient to treat it, mainly when there is mild infection like flu and cold. Some of the remedies are,

- Fluid intake should be increased.
- If the fever is high then bath to reduce it and not to eliminate it, room should be well ventilated.
- If the fever raises continuously then sponging with lukewarm water can be useful.
- Any type of stress should be avoided.
- Herbs and other cooking ingredient are also used to reduce fever.
- Boiled filtrate of basil leaves in water on drinking once a day reduces temperature.
- When fever is present then half teaspoon of saffron is taken with warm water.
- Grape fruit juice with water is useful in reducing fever.
- Honey mixed with ginger is useful in fighting fever. [23]

ALLOPATHIC TREATMENT OF FEVER

For treatment of fever it is necessary to know the fever's underlying cause. Different over the counter are used in reducing body temperature to a normal level as well as to treat the underlying cause. OTC antipyretic that are used normally includes nonsteroidal anti-inflammatory drugs like aspirin, nimesulide, paracetamol, ketoprofen, ibuprofen, meloxicam, celecoxib, rofecoxib etc.

TARGET OF ANTIPYRETIC DRUG

Cyclooxygenase (COX) enzyme is also known as prostaglandin endoperoxide synthase, and it is the key enzyme required for the synthesis of prostaglandins from arachidonic acid. Two COX isoforms that have been identified are COX-1 and COX-2. In many situations, the COX-1 enzyme is produced constitutively whereas COX-2 is highly inducible. Nonsteroidal anti-inflammatory drugs inhibit both COX-1 and COX-2 enzymes, while a new class of COX-2 selective inhibitors preferentially inhibits the COX-2 enzyme only. [23]

The elevated body temperature is reduced by antipyretic drugs which inhibit COX-2 expression thereby inhibiting prostaglandin synthesis. However these synthetic antipyretic agents inhibit the COX-2 with high selectivity but they have toxic effects on other organs like glomeruli, cortex of the brain, hepatic cells and heart muscles. [24]

Table 4: Adverse effect associated with NSAID Therapy [26]

System	**Adverse effect**
Gastrointestinal	Peptic ulcer Esophagitis and strictures Small and large bowel erosions
Renal	Reversible acute failure Chronic renal failure Interstitial nephritis Nephrotic syndrome Fluid and electrolyte disturbances
Cardiovascular	Exacerbation of hypertension Exacerbation of congestive cardiac failure Exacerbation of angina
Hepatic	Elevated transaminases Fulminant hepatic failure (rare)

CNS	Headache Drowsiness Confusion and behaviour disturbance Aseptic meningitis
Hematological	Thrombocytopenia Hemolytic anaemia Agranulocytosis and aplastic anaemia
Other	Exacerbation of asthma and nasal polyposis, Rash

2. REVIEW OF LITERATURE

- **Rafik U. Shaikh *et al.*, (2016)** investigated *in vivo* and *in vitro* anti-inflammatory potential of the extracted plant samples of *Cissus quadrangularis, Plumbago zeylanica, Terminalia bellarica* and *Terminalia chebulla* in water, ethanol and hexane. These were evaluated *in-vitro* for COX-1 and 2 inhibitory and antioxidant activities. Carrageenan model and Phorbol Myristate acetate induced mice edema model were used for the evaluation of *in vivo* anti-inflammatory activity. Indomethacin (20 mg/kg, p.o) was used as standard drug for carrageenan induced inflammation. Phorbol Myristate Acetate induced mice edema model was done by using indomethacin (2 mg per ear in 20 ml acetone) as standard. The results obtained revealed that most of the plants were found to inhibit COX-2 activity as compared to COX-1. The ethanolic extract showed effective DPPH, hydroxyl and superoxide radical scavenging activity. *In vivo* anti-inflammatory study shows that, *Terminalia bellarica* and *Terminalia chebulla* had a significant impact on inhibition of edema formation.[27]

- **Anum khan *et al.*, (2015)** evaluated antimicrobial, anti-inflammatory and antipyretic activity of *Chorisia speciosa* leaves. Antipyretic and anti-inflammatory activities of plant were determined by using Wistar albino rats. Rats were tested with doses of 200 and 400 mg/kg body weight (p.o). Antibacterial and antifungal activity of *Chorisia speciosa* were evaluated by disc diffusion method. Significant antibacterial activity was shown against *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa,* while methanol extract of *C.speciosa* showed noticeable zone of inhibition (11 mm) against fungal *Candida albicans* strain. In Carrageenan-induced rat hind paw oedema, inflammation was significantly antagonized by extracts of *Chorisia speciosa* at 400 mg/kg. Diclofenac sodium (10 mg/kg) was used as standard. In brewer's yeast induced pyrexia chloroform extract of *Chorisia speciosa* demonstrated dose dependently protection which is comparable to standard drug paracetamol (150 mg/kg). [28]

- **Vinodhini Velu *et al.*, (2015)** aimed to assess the phytochemical analysis, *in vitro* anti-arthritic activity and hemolytic activity of the petroleum ether, chloroform, ethyl acetate and aqueous extracts of *Tragia involucrate*. The phytochemical analysis revealed the presence of phytoconstituents such as phenols, flavonoids, sterols and terpenoids etc. The maximum inhibition of anti-arthritic activity by protein denaturation inhibition assay was observed in chloroform extract at the concentration of 200 µg/ml. And the extracts was also found to be non-hemolytic against the red blood cell membranes. [29]

- **Dr. S. Chitra *et al.*, (2015)** studied the anti-inflammatory activity of Kandangkathiri Kirutham at the dose of 100 mg/kg and 200 mg/kg. Anti-inflammatory activity was evaluated by Carrageenan-induced paw edema in rats using Diclofenac sodium as the standard drug (50 mg/kg). Treatment with Kandangkathiri Kirutham at the dose of 100 mg/kg shown displacement value ranges from 0.331 to 1.287 ml. Treatment at the dose of 200 mg/kg shown displacement value ranges from 0.355 to 1.237 ml. Treatment with standard drug Diclofenac at the dose of drug at 50 mg/kg shown displacement value ranges from 0.325 to 1.168 ml. From the result, it was concluded that test sample significantly reduced the paw edema induced by carrageenan at both the dose level. [30]

- **Mohammad Asim Khan *et al.*, (2015)** conducted the antipyretic study on crude (600 mg/kg and 1200 mg/kg) and aqueous extract (162 mg/kg and 324 mg/kg) of Gule Ghaafis on yeast induced pyrexia in animal model. The crude form of Gule Ghaafis showed significant reduction in fever in higher dose and the extract of Gule Ghaafis in both lower and higher dose reduced fever by inhibition of the production of prostaglandins. [31]

- **Anitha John *et al.*, (2015)** investigated Kabasura kudineer choornam by analytical methods and chromatographic studies with a view to suggest standards for evaluating its quality and purity. The choornam was subjected to physico-chemical analysis, preliminary phytochemical analysis, TLC and HPTLC studies. The preliminary phytochemical investigations of choornam showed the presence of

major secondary metabolites which reveals the potent therapeutic activity. TLC and HPTLC study indicated that the chemical constituents are present in significant quantity in the crude extract. [10]

- **Thillaivanan. S *et al.*, (2015)** reviewed the ethno pharmacological activities of the ingredients of Kabasura kudineer choornam to strengthen the scientific facts favoring this formulation. The phyto chemical constituents and pharmacological actions of the ingredients present in the formulation have anti-inflammatory, antipyretic, analgesic, anti-viral, anti-bacterial, anti-fungal, anti-oxidant, hepato protective, anti-diabetic, anti-asthmatic, anti-tussive, immunomodulatory, anti-diarrhoeal and anti-oxidant activities. Based on the survey the choornam can be used for preventative as well as curative for swine flu. [11]

- **T. Chandrasekar *et al.* (2015),** studied the medicinal efficacy of Nimbadipatra choornam by undertaking phytochemical analysis, antimicrobial activity effect, antioxidant effect and GC MS analysis. The phytochemicals present in choornam were saponins, tannins, triterpenoids, cardiac glycosides, phytosterol, coumarin and phenolic compounds. Strong antimicrobial activity of this medicine was observed against the microorganisms such as *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli* and *Candida albicans*. The potential of the choornam to serve as antioxidants were assayed by reducing power assay, peroxidase assay and catalase assay. The results of antibacterial, antifungal, antioxidant and GC MS analysis clearly indicate that the components present in choornam have important properties such as anti-viral, antibacterial, antifungal, antioxidant, anti-inflammatory etc. [32]

- **Ranveera Chathuranga Barathee *et al.*, (2014)** evaluated *in vitro* antiarthritic activity of aqueous root extract of *Clitoria ternatea* by using an *in vitro* bioassay model i.e. inhibition of heat induced denaturation of albumin protein. The antidenaturation action of aqueous root extract was found comparable to the reference drug Diclofenac sodium. From the observations it was concluded that the aqueous root extract of *Clitoria ternatea* possesses anti-arthritic activity. [33]

- **Ngoci Njeru S., (2014)** screened for the antibacterial activity of methanolic root extract of *Cissampelos pareira* L. by disc diffusion method. The highest inhibition was demonstrated toward *Staphylococcus aureus* (20 mm), *Pseudomonas aeuroginosa* (17 mm), *Klebsiella pneumoniae* (14 mm) and *Escherichia coli* at (9 mm). *Proteus vulgaris and Streptococcus pneumoniae* were not sensitive to the extract at all. The phytochemical screening demonstrated the presence of alkaloids, flavonoids, tannins, terpenoids and steroids. [34]

- **Evan Prince Sabina *et al.*, (2013)** evaluated the analgesic, antipyretic and ulcerogenic effects of piperine, which is an active ingredient of pepper. Mice were administered piperine (20 and 30 mg/kg) intraperitoneally. The analgesic activity of piperine were investigated by hot plate reaction test and acetic acid test. Antipyretic activity was determined by brewer's yeast induced hyperpyrexia model. It was found that piperine exhibits significant analgesic and antipyretic activities without any ulcerogenic effects. The results were comparable with indomethacin (10 mg/kg) which was used as standard drug for reference. [35]

- **Ismail Shareef. M *et al.*, (2013)** evaluated the anti-rheumatic properties of methanolic extracts of the aerial parts and roots of the plant *Clerodendrum serratum* L. (200 mg/Kg and 400 mg/Kg). The activity of the methanolic extracts was studied based on the effects of carrageenan-induced rat paw oedema by using the standard drug indomethacin (10 mg/Kg). The methanolic extracts of aerial parts and roots of *Clerodendrum serratum* L. produced significant anti-rheumatic activity in a dose-dependent manner. [36]

- **Sahaya Sathish S *et al.*, (2013)** investigated the antimicrobial activity of stem and leaf extract of *Tragia involucrata* L. Activity was determined against 7 pathogenic bacteria and fungi by disc diffusion method. The chloroform stem extract and ethanol leaf extract showed higher inhibition in bacterias like *Pseudomonas aeruginosa* and *Vibrio cholerae*. Ethanol and methanol stem extract has more effect against fungi like *Aspergillus niger* and *Rhizopus arrhizus*. There was no activity against *Penicillium chrysogenum.* [37]

- **Indira Priya Darsini *et al.*,(2013)** investigated the antibacterial properties of organic solvent (Ethanol, Methanol, Hexane) and water extracts of the whole plant of *Clitoria ternatea* against *Salmonella typhimurium, Proteus vulagaris, Shigella dysenteriae* and a fungal pathogen *Candida albicans* by agar disc diffusion method. Antibacterial activity was found to be more in methanolic extract. While others also proven antimicrobial activity. [38]

- **Bairagi Shripad M. *et al.*, (2012)** investigated for the analgesic and anti-inflammatory activities of *Ficus microcarpa* L. leaves extract in animal models. The extract at 50, 100 and 200 mg/kg reduced significantly the formation of oedema induced by carrageenan and histamine. In the acetic acid-induced writhing model, the extract showed a good analgesic effect characterized by a reduction in the number of writhes when compared to the control. Similarly, the extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin. These results were also comparable to those of diclofenac sodium (20 mg/kg), the reference drug used in this study. [39]

- **Agbaje E. O. *et al.*, (2012)** explored the anti-inflammatory potential of aqueous root extract of *Strophanthus hispidus* in rodents. Doses of 50, 100, 500 and 1000 mg/kg of aqueous extract were administered orally in carrageenan-induced hind paw oedema in rat, xylene induced ear oedema, and formalin-induced hind paw oedema in mice, using indomethacin (10 mg/kg), dexamethasone 1 mg/kg and acetylsalicylic acid 100 mg/kg respectively as standard drugs. Finally the results obtained showed that the extract exerted a dose-dependent and significant anti-inflammatory activity, which compared favourably with the positive control. [40]

- **Vasu Kandati *et al.*, (2012)** evaluated the analgesic and anti-inflammatory activity of both chloroformic and methanolic root extracts of *Andrographis serpyllifolia*. Chloroform and methanolic root extracts showed moderate potency in inhibiting 5-LOX. However, *in-vivo* anti-inflammatory studies revealed that methanolic extract has shown higher degree of efficacy when compared to the chloroformic

extract. In terms of analgesic activity, methanolic extract showed more efficacy than chloroformic extract. [41]

- **Anupama A Suralkar *et al*., (2012)** investigated the effects of methanolic extract of *Tamarindus indica* seeds on anti-inflammatory and analgesic activities *in vivo* using rat as an animal model at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight. The anti-inflammatory activities were investigated by utilizing carrageenan induced paw edema and the analgesic activity by tail immersion method in rats. The results showed that *Tamarindus indica* significantly reduced carrageenan induced paw edema in rats. In tail immersion method, extract showed significant increase in reaction time of tail in water maintained at 55°C indicating analgesic activity.[3]

- **Hemayet Hossain *et al*., (2012)** determined total flavonoids, tannin contents and performed anti-inflammatory activity of ethanolic root extract of *Lagenaria siceraria* at different doses (200 and 400 mg/kg). The percentage inhibition of the oedema paw volume by the 400 mg/kg body weight of the extract was statistically significant compared favorably with the indomethacin (10 mg/kg) treated animals at 1, 2 and 3 h in both carrageenan and histamine models. The total flavonoids and tannins content were calculated as quite high in ethanolic extract. [42]

- **Naveed Muhammad *et al*., (2012)** investigated antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. In yeast induced pyrexia, plant demonstrated dose dependently (78.23%) protection at 300mg/kg, similar to standard drug, paracetamol (90%) at 150 mg/kg i.p. plant also showed a dose dependent analgesia in various pain models i.e. acetic acid, hot plat and tail immersion at 300 mg/kg. Similarly carrageenan and histamine induces inflammation was significantly antagonized by plant, 66.30% and 60.80% respectively at 300mg/kg. Diclofenac (10 mg/kg) was used as standard for both study.[43]

- **Anbarasu K *et al*., (2011)** tested the antipyretic, anti-inflammatory and analgesic activity of ethanolic extract of nilavembu kudineer choornam (EENKC) to support its actions. In brewer's yeast induced pyrexia and carrageenan-induced inflammation, extract at the doses of 200 and 400 mg/kg inhibited fever and inflammation significantly compared to control groups. In mice, the number of writhing induced by acetic-acid was significantly reduced after treatment with both the dose of aqueous extract than control animals. And proved that choornam had antipyretic, anti-inflammatory, and analgesic activity. [44]

- **Paval et al., (2011)** demonstrate the antiarthritic activity of ethanol extract of *Tinospora cordifolia*. Arthritis was induced in Wistar rats using Freund's complete adjuvant and Bovine type II collagen. Various haematological parameters used to assess the effectiveness of the treatment. These were supported by histological study of the affected ankle joints using haematoxylin and eosin. Results suggested that oral dosage of 150 mg/kg body weight exhibited anti-arthritic activity in Freund's adjuvants arthritic rats. In collagen induced arthritic rats, the dosage of 100 mg/kg body weight showed anti-arthritic activity. [45]

- **Uma Chandur *et al*., (2011)** evaluated the presence of chemically active compounds of the petroleum ether, chloroform, and alcoholic extracts of the *Saussurea lappa* roots by standard methods and screened for their antiarthritic activity by using Complete Freund's Adjuvant (CFA) induced rat model. Application of all the three extracts exhibited significant edema inhibition when compared with the arthritic control group. The application of standard piroxicam gel was found to inhibit this edema to an extent of 66.96 %. The test extracts petroleum ether, chloroform and alcoholic application showed 66.08, 57.10 and 42.60 % inhibition respectively. [46]

- **N. Shekhawat *et al*., (2010)** investigated the crude methanolic extract of *Madhuca indica* at 50, 100 and 200 mg/kg for anti-inflammatory, analgesic and antipyretic activities in male Wistar rats. Anti-inflammatory activity was studied by using carrageenan-induced oedema right hind paw volume, the analgesic effect was

evaluated by acetic acid-induced abdominal pains and the brewer's yeast-induced pyrexia model was used for antipyretic investigation. All the doses of the plant methanolic extract and the indomethacin significantly inhibited carrageenan-induced inflammation that was not dose-dependent. The plant extract reduced the acetic acid-induced pain licking. The plant extract also reduced the brewer's yeast provoked elevated body temperature in rats after 60 min for 50 and 100 mg/kg dose range and 30 min for 200 mg/kg dose range. [47]

- **Aiyalu Rajasekaran *et al*., (2010)** conducted antipyretic activity on ethyl acetate extract of *Adenema hyssopifolium* in a rat model. It shows significant reduction in normal body temperature and brewer's yeast induced pyrexia in rat on a dose dependent manner. The antipyretic activity was compared with standard drug paracetamol (150 mg/kg). At the dose of 600 mg/kg it showed significant reduction in temperature in pyrexia rats from 2 to 4 h and 300 mg/kg shows significant reduction in temperature at 3 and 4 h. Flavonoids were reported for its antipyretic action and iridoid glycoside for its analgesic and antiphlogistic activity. [48]

- **G.Vinothapooshan *et al*., (2010)** investigated the methanolic, chloroform and diethyl ether extracts of *Adhatoda vasica* plants for its wound healing activity in the form of ointment dosage form in excision wound model in albino rats. The methanolic extract ointment of *Adhatoda vasica* showed a significant effect in excision wound model as comparable to standard drug (0.2 % w/w Nitrofurazone ointment) and other two extracts of ointment, by calculating the parameters, percentage closure of excision wound model. [49]

- **Sandeep biradar *et al*., (2010)** investigated the anti-inflammatory, anti-arthritic, analgesic and anticonvulsant activity of essential oils of *Cyprus esculentus* and *Cyprus rotundus*. The oils were evaluated for carrageenan induced inflammation, formaldehyde induced arthritis, formalin induced writhing and MES produced convulsion. The results showed dose dependent activity indicated by reduction in paw edema in anti-inflammatory and antiarthritic activity, and significant reduction in the MES induced convulsion in comparison to control. [50]

- **Anand Bafna *et al.*, (2010)** screened the alkaloidal fraction of roots of *Cissampelos pareira* Linn. (AFCP) for *in-vitro* antioxidant activity and immunomodulatory activity in mice. The HPTLC finger print profile was also established for the identification of AFCP which was found to contain 0.176 % of berberine. The potential of the extract to serve as antioxidants were assayed by DPPH, superoxide radical scavenging activity and by inhibiting lipid peroxidation in rat liver homogenate induced by iron/ADP/Ascorbate complex. [51]

- **Kumar *et al.*, (2009)** evaluated the anti-inflammatory activity of *Piper longum* fruit oil using different doses 0.5 ml/kg and 1 ml/kg orally by using carrageenan-induced paw edema model. The anti-inflammatory activity was dose dependant and found to be statistically significant at the higher concentration to that of ibuprofen (100 mg/kg) a standard reference drug. [52]

- **S. S. Bhujbal *et al.*, (2009)** examined the antioxidant effects of ethanolic extract of roots of *Clerodendrum serratum* at various concentrations in the DPPH radical scavenging assay, FRAP assay and the Hydrogen peroxide radical scavenging assay. The results revealed that the plant extract has significant antioxidant activity. The preliminary phytochemical investigation indicates the presence of flavonoids in the plant. So, the antioxidant potential of the plant may be attributed to the presence of flavonoids.[53]

- **Y Tanko *et al.*, (2008)** tested anti-nociceptive and anti-inflammatory effects by using the ethanolic extracts of *Syzygium aromaticum* flower bud. Acetic acid-induced abdominal contractions in mice and formalin-induced hind paw edema in wistar rats were the animal models used. The extract had an LD_{50} of 565.7 mg/kg body weight intraperitoneally in mice. Three doses of the ethanol extract (50, 100, and 200 mg/kg) i.p. produced significant effect in both the models. The result supports the local use of the plant in painful and inflammatory condition. [54]

- **Radhika P. *et al.*, (2008)** tested *in vitro* antibacterial and antifungal activity of hexane and methanolic extracts of the roots of *Andrographis paniculata*. The

extracts were found to inhibit the growth of all the bacteria and fungal organisms tested. The inhibition produced by the extracts were comparable with the benzyl penicillin, standard antibacterial agent and with the fluconazole, standard antifungal agent. [55]

- **G. Amresh *et al.*, (2007)** evaluated 50 % ethanolic extract of the aerial part of *Cissampelos pareira* for anti-inflammatory and analgesic activity in rats and mice respectively. Anti-inflammatory activity was investigated by carrageenan induced inflammation and arachidonic acid test. Whereas analgesic action of extract was evaluated through abdominal writhes and hot plate test. Oral administration of extract exhibited significant and dose dependent anti-inflammatory activity in the carrageenan test, which was based on interference with prostaglandin synthesis, as confirmed by the arachidonic acid test. In the abdominal writhing test induced by acetic acid, 200 mg/kg had the highest analgesic activity, whereas in the hot-plate test the best dose was 100 mg/kg.[56]

- **D. K. Pal *et al.*, (2006)** studied *in vitro* antioxidant activity of the roots and rhizomes of *Cyperus rotundus*. It has been investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520 nm. The ethanolic extract of the roots and rhizomes of *Cyperus rotundus* showed higher activity, than other extracts of it. The antioxidant activity of the extracts are close and identical in magnitude, and comparable to that of standard antioxidant compounds used. The result obtained, indicates that the ethanolic extract has more antioxidant activity than the petroleum ether, chloroform, and aqueous extract. [57]

- **B. Parimala Devi *et al.*, (2003)** evaluated the anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root against Carrageenan-induced rat paw oedema and vascular permeability induced by acetic acid models in rats respectively. The extract of root of *Clitoria ternatea* exhibited a significant inhibition of the oedema by 21.6 % and 31.8 %, at 200 and 400 mg/kg respectively. *Clitoria ternatea* also showed significant activity for both analgesic and antipyretic model. It was concluded that the methanol extract of *Clitoria ternatea* possesses significant anti-inflammatory, analgesic and antipyretic activities. [58]

- **Hua-Yew cheng *et al*., (2003)** investigated 6 extracts and 4 pure compounds of *Terminalia chebula* for anti-lipid peroxidation, anti-superoxide radical formation and free radical scavenging activities. The superoxide radical scavenging of the 4 pure compounds was further evaluated using electron spin resonance (ESR) spectrometry. The results showed that all tested extracts and pure compounds of *Terminalia chebula* exhibited antioxidant activity at different magnitudes of potency. [59]

- **S. C. Penna *et al*., (2003)** investigated the effects of crude hydroalcoholic extract of ginger rhizomes on rat paw and skin edema. The carrageenan, compound 48/80 or serotonin induced rat paw edema were inhibited significantly by i.p administration of alcoholic ginger extract. Ginger extract was also effective in inhibiting 48/80 induced rat skin edema at doses of 0.6 and 108 mg/site. Rat skin edema induced by substance P or bradykinin was not affected by treatment with ginger extract. The i.p administration of extract (186 mg/kg body wt.) 1 h prior to serotonin injections, reduced significantly the serotonin induced rat skin edema. Thus the crude extract was able to reduce the rat paw and skin edema induced by carrageenan, 48/80 compound and serotonin. [60]

3. AIM AND OBJECTIVE

3.1. AIM

Kabasura kudineer choornam, is a siddha formulation. It is commonly used for the treatment of fever with or without respiratory infection.

The aim of current study is to assess the anti-inflammatory, antipyretic and antibacterial effect of aqueous extract of Kabasura kudineer choornam (AEKKC).

3.2. OBJECTIVE

The objectives of the present study include:

- Evaluation of *in vitro* antioxidant activity of AEKKC
- Acute toxicity study of AEKKC
- Evaluation of anti-inflammatory, antipyretic and antibacterial activity of AEKKC

4. PLAN OF WORK

The present study examines the efficacy of **Kabasura kudineer choornam**, a siddha formulation. The effect of the drug was evaluated on various animal models, which was designed as follows.

1. Literature search
2. Selection of formulation
3. Aqueous extraction of Kabasura kudineer choornam
4. Preliminary phytochemical analysis & chromatographic screening
5. Quantification of total phenol and flavonoid content
6. *In vitro* antioxidant activity
    a. DPPH radical scavenging assay
    b. ABTS radical scavenging assay
7. Acute toxicity studies
8. Pharmacological Study

✓ Screening of anti-inflammatory activity
    a. Carrageenan induced inflammation
    b. Histamine induced inflammation

✓ Screening of antipyretic activity
    a. Brewer's yeast induced pyrexia model

9. Evaluation of antibacterial activity
10. Statistical analysis
11. Documentation of results

5. MATERIALS AND METHODS

5.1. MATERIALS USED FOR THE STUDY

Table 5: List of instruments

Sl. No	Instruments	Manufacturer
1	Analytical weighing balance	Shimadzu
2	Hot air oven	Narang scientific works
3	HPTLC	Camag
4	Plythesmometer	NIVIQURE
5	UV spectrometer	Pharmaspec UV-1700, Shimadzu
6	Electric water bath	Technico
7	Digital Thermometer	Sun pharmaceutical

Table 6: List of Chemicals

Drugs	**Chemicals**	**Others**
Diclofenac Paracetamol	2, 2'- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) 1,1- diphenyl-2-picrylhydrazyl (DPPH) Carrageenan Brewer's Yeast Histamine Quercetin Gallic acid Rutin Folin-Ciocalteau reagent Phosphate Buffer Potassium Persulfate Sodium Carbonate 10 % Aluminium Chloride 1 M Potassium acetate	Distilled water Sterile water for injection Normal saline Syringe Needle Gloves Whatmann No.1 filter paper

5.2. FORMULATION PROFILE

5.2.1. NAME

Kabasura kudineer choornam

5.2.2. DESCRIPTION

Decoction is prepared, which consist of water soluble active principles.

5.2.3. INDICATION

Fever

5.2.4. DOSE

Take 5 g of the powder mix it with 300 ml of water, boil and reduce to 30 ml and give it by addition with honey three to four times a day or as directed by the physician.[61] [11]

5.2.5. INGREDIENTS OF KABASURA KUDINEER CHOORNAM

Table 7: Ingredients of Kabasura kudineer choornam

Sl. No	Ingredients	Parts
1	*Zingiber officinale*	Rhizome
2	*Piper longum*	Fruit
3	*Syzygium aromaticum*	Bark, Bud
4	*Tragia involucrate*	Root
5	*Anacyclus pyrethrum*	Root
6	*Hygrophilla auriculata*	Root
7	*Terminalia chebula*	Fruit
8	*Adathoda vasica*	Leaf
9	*Coleus ambonicus*	Leaf
10	*Saussurea lappa*	Root
11	*Tinospora cardifolia*	Stem
12	*Clerodendron serratum*	Root
13	*Andrographis paniculata*	Whole plant
14	*Cissampelos pareira*	Root
15	*Cyperus rotandus*	Rhizome

5.2.6. DESCRIPTION OF INDIVIDUAL INGREDIENTS:

Zingiber officinale*:* In the fresh ginger rhizome, the gingerols were identified as the major active components. The powdered rhizome contains fatty oil, protein, carbohydrates, crude fiber, ash, water and volatile oil. In dried ginger powder, shogaol is a predominant. *Zingiber officinale* is non-toxic, highly promising natural antioxidant compound having a wide spectrum of biological function such as antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, anticarcinogenic activity etc. [62]

Piper longum*:* Decoction of immature fruits and roots of *piper longum* were used in chronic bronchitis, cough and cold. Roots and fruits were also used as anti-dote in snake biting and scorpion sting. It also shows insecticidal and acaricidal, antifungal, anti-amoebic, antimicrobial, antiasthmatic, antidiabetic, hypocholesterolaemic, antioxidant, analgesic, anti-inflammatory, anti-depressant activity. Piperine is the major constituent. Piperine showed marked increase in serum gonadotropins and a decrease in intratesticular testosterone concentration, despite normal serum testosterone titres. The fruits also gave positive tests for the presence of volatile oil, starch, protein and alkaloids, saponins, carbohydrates, and amygdalin. [63]

Syzygium aromaticum: Used for the treatment of asthma and various allergic disorders. It was used ad general antiseptic in medical dental practices. Importantly, due to its antioxidant properties clove oil might also be used as an anti-carcinogenic agent. The buds oil mainly contains eugenol (49.71%), caryophyllene (18.94%), benzene, 1-ethyl-3- nitro (11.12%), benzoic acid, 3-(1-methylethyl) (8.95%), elixene (3.87%), caryophyllene oxide (1.53%) and - farnesene (1.11%). [64]

Tragia involucrate: The roots are bitter, acrid, sweet, cooling possessing diuretic, diaphoretic, antiperiodic, depurative and alterant activity. They are useful in pruritic skin eruptions, venereal diseases, haemorrhoids, gastropathy, guinea worms, blood impurities, dyspepsia, vomiting giddiness, vitiated conditions of pitta, melalgia and brachialgia. It is also useful when the extremities are cold during fever and also for pains in the legs and arms. The root forms the basis of an external application in leprosy, in old venereal complaints and a blood purifier.[65] It shows the presence of alkaloids, carbohydrates, protein, tannins, flavonoids, sterols and saponins. And also contains the presence of several

colorless phytocompounds such as vinyl hexylether, shellsol, 2, 4 -dimethyl hexane, 2-methylnanone, and 2, 6-dimethyl heptane. [66]

***Anacyclus pyrethrum*:** Shows several pharmacological activities and medicinal properties like anti-rheumatic, analgesic, antibacterial, antiviral, carminative, anti-catarrhal, emmenagogue, febrifuge, nervine, vermifuge, and sialagogue activity. Various pharmacological actions such as antidiabetic, immunostimulating effect, inhibitory effects, antidepressant activity, anticonvulsant activity, memory-enhancing activity, action on COX and LOX has been reported for the plant. Various secondary metabolites such as alkaloids, reducing compounds, tannins, flavonoids and coumarins has been identified. Chemical analysis of roots shows the presence of three fatty acids, a sterol and ten unsaturated amides. The most important compounds discovered in roots are pellitorin, anacyclin, phenylethylamine, inulin, polyacetylenic amides I-IV, and sesamin. [67]

Hygrophila auriculata: Leaves are tonic, hypnotic. *Hygrophila auriculata* leaves are useful in dysentery, thirst, urinary calculi, urinary discharges, inflammations, biliousness, anaemia, constipation, anuria, cough and pains in the joints. Seeds are cooling, tonic, aphrodisiac, sedative to gravid uterus and constipating. It can be given for gonorrhoea and spermatorrhoea. Decoction of the root is used as a diuretic in dropsy. The plant is used in cancer and tubercular fistula EtOH (50%) extract of plant is spasmolytic and hypotensive. Aerial parts contains alkaloids, phytosterol, lupeol, stigmasterol etc. The seeds contain asterol I, II, III and IV and asteracanthine & asteracanthicine. Apigenin glucuronide is isolated from the flowers. And Roots contain an essential oil. Palmitic, stearic, oleic and linoleic 80.1%) acids have been detected in seed oil. [68]

Terminalia chebula: *Terminalia chebula* contains 32% of tannin. *Terminalia chebula* are of pyrogallol type. They contain 14 components of hydrolysable tannins (gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic, ellagic acid, chebulegic acid, chebulinic acid , 1, 2, 3, 4, 6 -penta -O galloyl-ß-D-glucose, 1, 6,-di-O-galloyl-D-glucose, casuarinin, 3, 4, 6-tri-O-galloyl-D-glucose and terchebulin). Flavonol glycosides, triterpenoids, chebulin, as well as phenolic compounds were also isolated. In addition, ethyl gallate and luteolin were also isolated from the fruit of *Terminalia chebula*. In Unani system, it is used as a blood purifier. The pulp of the fruit is given in piles, chronic diarrhea,

dysentery, costiveness, flatulence, asthma, urinary disorder, vomiting, hiccup, intestinal worms, ascites and enlarged spleen and liver. *Terminalia chebula* showed a melanin inhibitory effect higher than 90%. [69] [70] [71]

Adathoda vasica: The vast variety of pharmacological uses of Adhatoda is believed to be the result of its rich concentration of alkaloids. The prominent alkaloid found in Adhatoda leaves is vasicine, a quinazoline alkaloid. The leaves and roots of Adhatoda, in addition to vasicine, contains the alkaloids l-vasicinone, deoxyvasicine, maiontone, vasicinolone and vasicinol. These are responsible for adhatoda's bronchodilatory effect. Cholagogue activity, anti-tubercular activity, abortifacient and uterotonic activity, anti-allergy activity are some other pharmacological activity. [72]

Coleus aromaticus: It is known to possess antimicrobial, antiepileptic, leishmanial, and antioxidant activities. The leaves are also used for treatment of cough, throat infection and nasal congestion. [73]

Saussurea lappa: Costunolide is one of the major bioactive constituent of *Saussurea lappa* root. Traditionally it has been used for the treatment of large number of diseases such as asthma, cough, throat infections, tuberculosis, leprosy, malaria, convulsions, fever, helminthic infestations and as antispasmodic. And also reported pharmacological actions such as antiulcerogenic, cytotoxic, anti-inflammatory, anti-epileptic, antibacterial, hepatoprotective, immunomodulatory, anticonvulsant, angiogenesis, antidiarrheal, antihyperlipidemic activity etc. [74]

Tinospora cordifolia: The therapeutic properties of medicinal plants are attributed owing to the presence of active substances such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins. [75] From the stem of *Tinospora cordifolia* alkaloid berberin, tinosporin and palmitin were isolated. From the whole plant of *Tinospora cordifolia*, the diterpenoidal lactone tinosporide and tinosporon were obtained. In ayurvedic system of medicine it is widely used for general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, anti- arthritic, anti-allergic, antidiabetic properties and also to improve the immune system and the body resistance against infections. The stem is bitter, stomachic, diuretic, stimulates bile secretion, causes constipation, allays thirst, enriches the blood and cures jaundice. The extract of its stem is useful in skin problems. [76]

Clerodendrum serratum: Root reported the presence of flavonoids, glycosides, saponins, sterols and absence of alkaloids and terpenoids. The root bark contain D-mannitol. Its roots are bitter, acrid, thermogenic, anti-inflammatory, anthelmintic, depurative, expectorant, sudorific, antispasmodic, stimulant and febrifuge. The root also increases appetite, lessens expectoration. [77]Aqueous extracts of leaves of *Clerodendrum serratum* possess bronchodilator property. Previous studies suggests that apigenin-7-glucoside has demonstrated anti-inflammatory, antimicrobial, hepatoprotective and anti-diarrheal properties. The compound also showed significant protection against Alzheimer's disease in mice. [78]

Andrographis paniculata*:* Andrographolide is the major constituent. Some other known constituents are Neoandrographolide, Paniculide-A, Paniculide-B, Paniculide-C etc. [79] In China, India, Thailand and Malaysia, this has been widely used for treating sore throat, common cold, flu and upper respiratory tract infections. In Ayurvedic system of medicine it is used as carminative, liver stimulant, immune system stimulant, anthelmintic, blood purifier, anti-inflammatory, antipyretic, anti-malarial and in prevention of infections. In Siddha system of medicine it is used for treating cancer. *Andrographis paniculata* either alone or with neem or papaya juice/extract can use for the management of malarial fever, dengue fever, chikungunya and common flu. [80]

Cissampelos pareira: The roots are bitter astringent, carminative, digestive, anti-inflammatory, and pungent. And also shows diuretic, febrifuge, expectorant, galactagogue activity. It is useful in dyspepsia, indigestion, flatulence, abdominal pains, diarrhoea, dysentery, blood disorders, cardiac disorders, edema, leprosy, sensation, asthma, bronchitis, cystitis, dysuria and lactation disorders also. In addition it is useful in non-healing ulcers, skin disorders, scabies, migraine, leucorrhoea and gonorrhea. It mainly consists alkaloids, such as hayatine, hayatinine, hayatidine and other bisbenzylisoquinoline alkaloids. [81]

Cyperus rotundus: *Cyperus rotandus* are useful in treatment of fever, malaria, cough, bronchitis, renal and vesical calculi, skin diseases, wounds, amenorrhoea, dysmenorrhoea, loss of memory, insect bites, food poisoning, infertility, cervical cancer and menstrual disorders. And also useful for dietary management of psychotic diseases and metabolic

disorders. *Cyperus rotandus* rhizomes revealed the presence of alkaloids, flavonoids, tannins, starch, glycosides, furochromones, monoterpenes, sesquiterpenes, sitosterol, fatty oil containing a neutral waxy substance, glycerol, linolenic, myristic and stearic acids. The major compounds isolated from essential oil and the extracts of *Cyperus rotundus* rhizome are α-cyperone, α-rotunol, β-cyperone etc. [82]

5.3. METHODOLOGY

5.3.1. COLLECTION OF THE FORMULATION

Kabasura kudineer choornam was procured from Siddha Practioner Dr. K. Anbarasu, Trichy.

5.3.2. EXTRACTION OF THE FORMULATION [83]

Preparation of extracts: An aqueous extract of Kabasura kudineer choornam (100 gm) was prepared by heating the mixture at 50-60º C till water reduces to 1/8th of its volume. This procedure involves simple decoction process to obtain the soluble materials being extracted from the crude raw plants, which was then cooled and filtered. The filtrate that obtained by decoction process was then concentrated. The concentrated aqueous extract of Kabasura kudineer choornam (AEKKC) was stored at 2-5 °C until completion of study.

5.3.3. QUALITATIVE PHYTOCHEMICAL ANALYSIS [84] [85] [86] [87]

Preparation of test sample

500 mg of the extract was dissolved in 5 ml of distilled water and then filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample.

5.3.3.1. TEST FOR CARBOHYDRATES

- **Molisch's test**

A few drops of Molisch's reagent were added to 2-3 ml of filtrate, and concentrated sulphuric acid was added along the sides of the test tube. Violet colour ring formed at the junction of two liquids indicates the presence of carbohydrates.

- **Fehling's test**

Equal volume of Fehling's- A [copper sulphate in distilled water] and Fehling's- B [potassium tartarate and sodium hydroxide in distilled water] reagents were mixed in a test tube and boiled for one minute. 1ml of sample was added to the above mixture and heated for few minutes. Brick red precipitate formed confirms the presence of sugars.

- **Benedict's test**

The test sample was mixed with equal volume of Benedict's reagent (alkaline solution containing cupric citrate complex) in a test tube and heated for few minutes. Formation of brick red precipitate confirms the presence of sugars.

5.3.3.2. TEST FOR ALKALOIDS

Small amount of extract was mixed with few ml of dilute hydrochloric acid and filtered. The following tests were performed with the filtrate:

- **Dragendorff's test**

Few drops of the Dragendorff's reagent (Sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate) were added to 2-3 ml of filtrate. Development of orange brown precipitate indicates the presence of alkaloids.

- **Mayer's test**

The filtrate was treated with few drops of Mayer's reagent (mercuric chloride and potassium iodide). Formation of yellowish buff coloured precipitate indicates the presence of alkaloids.

- **Wagner's test**

A few drops of filtrate were treated with Wagner's reagent (solution of iodine in potassium iodide) and a reddish brown precipitate obtains to indicate the presence of alkaloids.

- **Hager's test**

A few drops of Hager's reagent were added to 2-3 ml of filtrate. Development of yellow precipitate indicates the presence of alkaloids.

5.3.3.3. TEST FOR TRITERPENOID

- **Libermann-Burchard test**

A small quantity of extract was treated with few drops of acetic anhydride, followed by a few drops of concentrated sulphuric acid. A brown ring was formed at the junction of two layers and the upper layer turns green colour, infers the presence of phytosterols and

formation of deep red colour indicates the presence of triterpenoids.

- **Salkowski test**

A small quantity of the extract was treated with chloroform and few drops of concentrated sulphuric acid and allowed to stand for few minutes. Yellow colour at the lower layer indicates the presence of triterpenoids.

5.3.3.4. TEST FOR GLYCOSIDES

- **Legal's test**

2 ml of extract was dissolved in pyridine. Sodium nitroprusside solution was added to it and made alkaline. Pink red colour formed indicates the presence of glycosides.

- **Keller-Killiani test**

2 ml of extract was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of two liquids, a reddish brown color formed, which gradually became blue colour due to the presence of glycosides.

- **Baljet test**

2 ml of extract was added to sodium picrate solution. Yellow to orange colour was formed indicating the presence of glycosides.

5.3.3.5. TEST FOR STEROIDS AND STEROLS

- **Liebermann- Burchard reaction**

The test sample was dissolved in 2 ml of chloroform in a dry test tube. Few drops of acetic anhydride were added followed by 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

- **Salkowski reaction**

Extract was dissolved in chloroform and concentrated sulphuric acid was added to it. Bluish red, cherry red and purple colour was noted in chloroform layer, whereas acid layer was marked with green fluorescence.

5.3.3.6. TEST FOR TANNINS

- **Lead acetate test**

1 ml of alcoholic solution of extract was diluted with 5 ml of distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow colour precipitate was formed which indicates the presence of phenols.

5.3.3.7. TEST FOR SAPONINS

- **Foam Test**

About 1 ml of test sample was diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 3 minutes. Foam of 1cm after 10minutes indicates the presence of saponins.

- **Froth test:**

A drop of sodium bicarbonate was added to 5 ml of the test sample. The mixture was shaken vigorously and kept for 3minutes. A honey comb like froth was formed which shows the presence of saponins.

5.3.3.8. TEST FOR PHENOLS

- **Ferric chloride test**

1 ml of the alcoholic solution of the extract mixed with 2 ml of distilled water followed by few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

- **Lead acetate test**

1 ml of alcoholic solution of extract was diluted with 5 ml of distilled water and, few drops of 1% aqueous solution of lead acetate was added. A yellow colour precipitate was formed which indicates the presence of phenols.

5.3.3.9. TEST FOR FLAVONOIDS

- **Alkaline reagent test**

A few drops of sodium hydroxide solution was added to the extract. Intense yellow colour turned to colourless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

- **Shinodas test [Magnesium hydrochloride reduction test**]

A small piece of magnesium ribbon and few drops of concentrated hydrochloric acid were added to the alcoholic solution of extract and heated. Appearance of crimson red or occasionally green to blue colour infers the presence of flavonoid.

5.3.3.10. TEST FOR PROTEINS AND AMINO ACIDS

- **Biuret test**

1 ml of test sample was mixed with 1 ml of 40 % sodium hydroxide and 2 drops of 1% copper sulphate. Formation of violet colour indicates the presence of proteins.

- **Ninhydrin test**

1 ml of test sample heated with 2 drops of freshly prepared 0.2% Ninhydrin reagent. Development of blue colour indicates the presence of proteins, peptides or amino acids

5.3.4. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY [88]

HPTLC has emerged as an important tool for the qualitative and semi quantitative and quantitative phytochemical analysis of herbal drugs. HPTLC has excellent resolution and therefore it permits simultaneous identification of several samples in a single run, using a small quantity of mobile phase. It is a high output, time saving and a rapid low cost analytical method.

Preparation of standard solution

Stock solutions of standard compounds were prepared by dissolving accurately weighed 1mg of Gallic acid, Rutin, Quercetin, Ferrulic acid, Andrographolide, and Ellagic acid in 1 ml of methanol (HPTLC grade). And 5 µl of each standard were spotted on the HPTLC plate.

Preparation of Sample

Accurately weighed 1 g of Kabasura kudineer choornam and dissolved separately in 10 ml methanol and water. Also weighed 1 g of AEKKC and dissolve in 10 ml water. Each sample was then filtered by using Whatmann No.1 filter paper. 10 µl of 3 samples were spotted on the HPTLC plate.

Experimental condition

Stationary phase	: Aluminium plates precoated with Silica Gel 60F254 (10×10×0.2) mm thickness
Mobile phase	: Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4)
Sample for HPTLC	: Kabasura Kudineer choornam, AEKKC, Standard Quercetin, Rutin, Gallic acid, Ferrulic acid, Andrographolide, Ellagic acid solutions.

Materials and methods

Sample application	: Camag Linomat 5
Chamber type	: Twin trough Chamber 10× 10 cm
Chamber saturation	: 5 min
Development time	: 30 min
Development distance	: 7 cm
Detection	: Camag Scanner 3
Data system	: win CATS Planar Chromatography Manager.

Instrumental Parameters

Number of track	: 9
Band length	: 6.0 mm
Application position	: 10 mm

Solvent front position : 80.0 mm

Solvent volume : 10 ml

Position of first track : 10 mm

Distance between tracks : 10 mm

Scan start position Y : 5.0 mm

Scan end position : 75.0 mm

Slit dimension : 6.00 × 0.45mm, Micro

Optimized optical system : light

Scanning speed : 20 mm/sec

Data resolution : 100 µM/ step

Measurement table

Wavelength : 254 nm

Lamp : D2 and W

Measurement : remission

Measurement mode : absorption

Optical filter : second order

Detector mode : automatic

Procedure

The standards; gallic acid, rutin, quercetin, ferrulic acid, andrographloide, ellagic acid (5µl) and samples; Kabasura Kudineer choornam in methanol, AEKKC in water and Kabasura Kudineer choornam in methanol (10 µl) were spotted in form of bands with a Camag microlite syringe on pre-coated Silica Gel glass plate 60F254 (10×10 cm with 0.2mm thickness) using a Camag Linomat 5 applicator. The plates were pre-washed with methanol and activated at 60^0 C or 10 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber after chamber saturation with

respective mobile phase. The optimized chamber saturation time for mobile phase was 5 min at room temperature. Linear ascending development was carried out and the plate was developed in the respective mobile phase up to 7 cm. The developed plate was then dried by hot air to evaporate solvents from plate and also for the development of bands. The dried plate was observed under UV light at 254 nm and 366 nm and photo documentation was done. Densitometric scanning was performed on Camag TLC scanner 3 in the absorbance mode at 280 nm. The percentage of active constituents present in the extract was compared with that of standard.

5.3.5. QUANTIFICATION OF TOTAL PHENOLICS AND FLAVONOIDS [89]

5.3.5.1. ESTIMATION OF TOTAL PHENOLICS

Reagents

Folin-Ciocalteu's reagent

Gallic acid (1mg/ml)

20% sodium carbonate

Preparation of standard

Standard solution was prepared by adding 10 mg of accurately weighed Gallic acid in 10 ml of distilled water.

Preparation of sample

10 mg of the accurately weighed extract was dissolved in 10 ml water and used for the estimation.

Procedure

The total phenolic content of the extract was determined by Folin-Ciocalteau assay method. To an aliquot 100 µl of extract (or standard solution of Gallic acid (10, 20, 40, 60, 80, 100 µg/ml) added 50 µl of Folin-Ciocalteau reagent followed by 860 µl of distilled water and the mixture is incubated for 5 min at room temperature. 100 µl of 20% sodium carbonate and 890 µl of distilled water were added to make the final solution to 2 ml. It was incubated for 30 min in dark to complete the reaction. The absorbance of the mixture

was measured at 725 nm against blank. Distilled water was used as reagent blank. The tests were performed in triplicate to get mean the values. The total phenolic content was found out from the calibration curve of Gallic acid. And it was expressed as milligrams of Gallic acid equivalents (GAE) per gram of extract.

5.3.5.2. ESTIMATION OF TOTAL FLAVONOIDS

Reagents

Methanol

10% aluminium chloride

1M Potassium acetate

Preparation of standard

Standard solution was prepared by adding 10 mg of accurately weighed quercetin in 10 ml of ethanol.

Preparation of sample

10 mg of the accurately weighed extract was dissolved in 10 ml ethanol and used for the estimation.

Procedure

The total flavonoid content of the AEKKC was determined by using Aluminium chloride colorimetric method. To an aliquot of 100 µl of extract or standard solutions of Quercetin (10, 20, 40, 60, 80, 100 µg/ml) ethanol was added separately to make up the solution upto 2 ml. The resulting mixture was treated with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. Shaken well and incubated at room temperature for 30 minutes. The absorbance was measured at 415 nm against blank, where a solution of 2 ml ethanol, 0.1 ml potassium acetate, 2.8 ml distilled water and 0.1 ml of aluminium chloride serve as blank solution. The total flavonoid content was determined from the standard Quercetin calibration curve and it was expressed as milligrams of Quercetin equivalents (QE) per gram of extract.

5.3.6. *IN VITRO*-ANTIOXIDANT STUDIES

5.3.6.1. DPPH FREE RADICAL SCAVENGING ASSAY [90]

Principle

The molecule of 1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol/methanol solution centred at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is Z• + AH = ZH + A•.

Procedure

The antioxidant activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical (Blois method).0.3 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 1 ml of various concentrations of sample (10, 20, 40, 60, 80 and100 µg/ml) and the reference compound quercetin (5, 10, 15, 20, 25 and 30 µg/ml) shaken vigorously and left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm against a blank. Reference compound used here was ascorbic acid. A control reaction was carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Antiradical activity was expressed as percentage inhibition (I %) and calculated using the following equation:

Percentage inhibition (I %) = (Abs $_{control}$- Abs $_{sample}$ /Abs $_{control}$) X 100

Different sample concentrations were used in order to obtain calibration curves and to calculate the IC_{50} values. (IC_{50} - concentration required to obtain a 50% radical scavenging activity).

5.3.6.2. ABTS FREE RADICAL SCAVENGING ASSAY [91]

In ABTS decolourization assay, the peroxidase substrate 2, 2'- azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS), forms a relatively stable radical ($ABTS^+$) upon one electron oxidation. This assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical and inhibits the absorption of the radical cation which has characteristic long-wavelength absorption spectrum showing maxima at 660, 734, and 820 nm. The relatively stable ABTS radical has a green colour and is quantified spectrometrically at 734 nm.

Procedure

ABTS radical scavenging activity of the extract was measured by Rice-Evans method. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12- 16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study, ABTS solution was diluted with phosphate buffer saline pH 7.4 (PBS) to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30 ^{0}C. After addition of 1 ml of diluted ABTS solution to various concentrations of sample (1, 2, 4, 6, 8, and 10 μg/ml) or reference compound (0.25, 0.5, 0.75, 1, 1.25 and 1.5 μg/ml) the reaction mixture was incubated for 6 min and then absorbance was measured at 734 nm against a blank. A control reaction was carried out without the sample. All the tests were performed in triplicate in order to get the mean values. The percentage inhibition of $ABTS^+$ by the sample was calculated according to the formula:

Percentage inhibition (I %) = ($Abs_{control}$- Abs_{sample} /$Abs_{control}$) X 100

Different sample concentrations were used in order to obtain calibration curves and to calculate the IC_{50} values. (IC_{50} - concentration required to obtain a 50% radical scavenging activity).

5.3.7. PHARMACOLOGICAL STUDY

ANIMALS

Male Wistar rats of 6-8 weeks old and 160-180 g body weight were offered by KMCH College of pharmacy, Coimbatore. All rats were housed and maintained under standard conditions of temperature (25 ^{0}C ± 5 ^{0}C), relative humidity (55 ± 10%), and 12/12 h light/dark cycle. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. Protocols for the study were approved by the Institutional Animal Ethical Committee (IAEC) for Animal Care and were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

5.3.7.1. ACUTE TOXICITY TEST

Acute oral toxicity study was performed as per OECD-423 guidelines. The mices were fasted overnight with free excess of water and were grouped into four group consisting of 3 animals each, to which the extract was administered orally at the dose level of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. They were observed for mortality; toxic symptoms such as behavioral changes, locomotor activity, convulsions; direct observation parameters such as tremor, convulsion, salivation, diarrhoea, sleep, coma, changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and CNS, somatomotor activity etc. periodically for 30 min during first 24 h. And specific attention given during first 4 hours daily for a total period of 14 days

5.3.7.2. SCREENING OF ANTI-INFLAMMATORY ACTIVITY

Experimental methods

Anti-inflammatory activity was assessed by carrageenan and histamine induced rat paw edema methods

- **CARRAGEENAN INDUCED PAW EDEMA IN RATS**

Experimental design

Wistar albino rats weighed around 150-250 g were used for the study. Rats were divided into four groups of 6 animals each.

Table 8: Experimental design for carrageenan induced paw edema

GROUPS	TREATMENT
Group I	Carrageenan 1% w/v (0.1 ml, subplantar)
Group II	Diclofenac (20 mg/kg), p.o
Group III	AEKKC (200mg/kg), p.o
Group IV	AEKKC (400mg/kg), p.o

Procedure [39] [92] [93]

The rats were divided into four groups of 6 animals each. The group II was treated with diclofenac (20 mg/kg) p.o and group III and IV were treated with 200 and 400 mg/kg of AEKKC respectively. Treatments were given 30 min before the administration of carrageenan. The rats were then challenged with subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan into the sub plantar region of left paw. The paw was marked with ink at the level of lateral malleolus. The paw volume was measured before (0 h) and after carrageenan injection at 1, 2, 3, 4, 5 h by volume displacement method using plythesmometer. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically. The percentage inhibition (PI) of paw edema was calculated by using the following formula;

Percentage of edema inhibition = $(V_c - V_t/V_c) \times 100$

V_c = Mean edema volume in control

V_t = Mean edema volume in group treated with standard or extract

- **HISTAMINE INDUCED RAT PAW EDEMA [39] [94]**

Experimental design

Wistar albino rats were divided into four groups of 6 animals each.

Table 9: Experimental design for histamine induced paw edema

GROUPS	TREATMENT
Group I	0.1 ml of freshly prepared histamine (0.1%)
Group II	Diclofenac (20 mg/kg), p.o
Group III	AEKKC (200 mg/kg), p.o
Group IV	AEKKC (400 mg/kg), p.o

Procedure

One hour after the drug treatment, inflammation was induced by injection of 0.1 ml of freshly prepared histamine (1%) in normal saline underneath the plantar tissue of the right hind paw of rats. Paw volume, measured using a plythesmometer before histamine administration and at 1, 2, 3 h after histamine injection. The percentage inhibition (PI) of paw edema was calculated by using the following formula;

Percentage of edema inhibition = $(V_c - V_t/V_c) \times 100$

V_c = Mean edema volume in control

V_t = Mean edema volume in group treated with standard or extract

5.3.7.3. SCREENING OF ANTIPYRETIC ACTIVITY

- **BREWER'S YEAST INDUCED PYREXIA MODEL [28]**

Experimental design

Wistar albino rats were divided into four groups of 6 animals each.

Table 10: Experimental design for brewer's yeast induced pyrexia model

GROUP	TREATMENT
Group I	10 ml/kg of 15% w/v Brewer's yeast suspension
Group II	Paracetamol 150mg/kg, p.o
Group III	AEKKC (200mg/kg), p.o
Group IV	AEKKC (400mg/kg), p.o

By insertion of a Digital thermometer to a depth of 2 cm into the rectum, the initial rectal temperatures were recorded. The fever was induced in animal by injection of 10 ml/kg of 15 % w/v Brewer's yeast suspension in normal saline subcutaneously in the back below the nape of the neck. The site of injection is massaged in order to spread the suspension beneath the skin. The room temperature kept at 22-24 ^{0}C. Immediately after Brewer's yeast administration, food is withdrawn. 18 h post challenge, the rise in rectal temperature was recorded. The measurement is repeated after 30 min. Only those rats which showed an increase in temperature of at least 0.6 ^{0}C (10 F) were used for further experiment. Fever induced rats were divided into four groups of six animals each. The animals were then received the test and standard drug by oral administration. After the drug administration the rectal temperature of all the rates in each group was recorded periodically at an interval of 1, 2, 3, 4, and 5 h of the drug administration. The percentage decrease in pyrexia was calculated by the following formula:

Percent reduction = $(B - C_n) / (B - A) \times 100$

B represents temperature after pyrexia induction;

C_n temperature after 1, 2, 3, 4 and 5 h

A is normal body temperature.

5.3.8. ANTI-BACTERIAL ACTIVITY [95] [96]

5.3.8.1. ZONE OF INHIBITION

Preparation of inoculums

The inoculums for the experiment were prepared in fresh Nutrient broth from the preserved slant culture. The turbidity of the culture can be adjusted by the addition of broth or sterile saline (if it is excessive) or by further incubation to get the required turbidity, and the newly prepared inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards.

Preparation of sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or by dry heat (only for the wooden swabs). It was sterilized by packing the swabs in culture tubes.

Sterilization of forceps

Forceps was sterilized by dipping in alcohol and burning off the alcohol.

Procedure

The standardized inoculums were inoculated in the sterilized plates prepared earlier (aseptically) by dipping a sterile loop into the inoculums. The excess inoculums was removed by passing and rotating the swab firmly against the side of the culture tube above the level of the liquid and streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed. The sterile discs were soaked overnight in sample solution. Each Petri dish was divided into 2 parts. First compartment of the plate were loaded with AEKKC disc (200 µg) and the second compartment with Std ciprofloxacin disc (10 µg) with the help of sterile forceps. After that the petri dishes are placed in the refrigerator at 4º C or at room temperature for 1 hour for diffusion. Incubate at 37 º C for 24 hours. Observe the zone of inhibition produced. Measure it using a scale and record the average of two diameters of each zone of inhibition.

Table 11: Bacterial strain used for the study with NCIM

Sl. No	Organism	Strain	NCIM
1	Gram $^{+ve}$ Bacteria	*Staphylococcus aureus*	2079
2		*Bacillus subtilis*	2063
1	Gram $^{-ve}$ Bacteria	*Pseudomonas aeuroginosa*	2200
2		*Escherichia coli*	2065

5.3.8.2. MINIMUM INHIBITORY CONCENTRATION

The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that prevents the visible growth of the organism i.e., the lowest concentration at which it has bacteriostatic activity.

Preparation of test drug:

The AEKKC was prepared in DMSO at a concentration 2000 µg/ml

Preparation of inoculum:

Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeuroginosa, Escherichia coli were the four strains of organisms selected for the study. Overnight culture are grown at 37 ^{0}C Kirby- Bauer procedure and diluted to Muller Hinton Broth. This overnight culture was diluted to 10^{-2}.

Inoculation:

The sterile tubes were labelled 1-8 and 8th tube was taken as control. 1 ml of Muller Hinton Broth was transferred to all tubes. 1 ml of drug solution was added to 1st tube and mixed well. From the 1st tube 1 ml of solution was transferred to the 2nd tube and was repeated up to 7th tube. From the final 2 ml volume of 7th tube 1 ml of solution was pipette out. 0.01 ml of culture was added to all the test tubes and all the tubes were incubated at 37 ^{0}C for 18-24 hrs. After incubation the turbidity was observed visually. The highest dilution without growth is the minimal inhibitory concentration.

5.3.9. STATISTICAL ANALYSIS

Data were analyzed by one way ANOVA followed by Dunnetts's multiple comparison test using Graphpad 5.0 software. The values were expressed as Mean ± SEM. $P<0.05$ was considered significant

6. RESULTS

6.1. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Table 12: Phytochemical analysis

Sl. No	Phytochemical constituents	AEKKC
1	Carbohydrates	Positive
2	Glycosides	Positive
3	Alkaloids	Positive
4	Phenolics	Positive
5	Flavonoids	Positive
6	Tannins	Positive
7	Triterpenoids	Positive
8	Saponins	Positive
9	Steroids	Positive
10	Proteins and amino acids	Negative

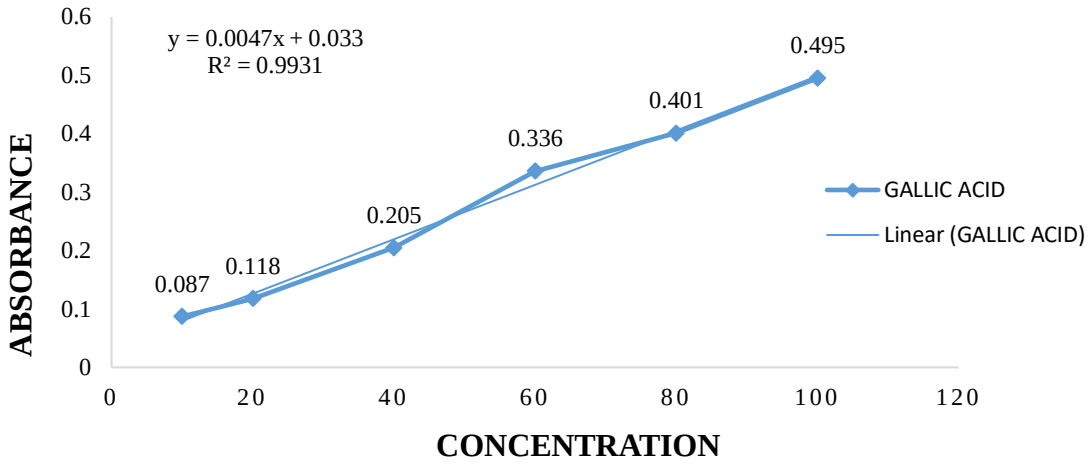
6.2. QUANTIFICATION OF TOTAL PHENOL AND FLAVANOIDS

6.2.1 ESTIMATION OF TOTAL PHENOL OF AEKKC

Table 13: Estimation of total phenolic content of AEKKC

Sample	Concentration (µg/ml)	Absorbance at 725 nm
Standard (Gallic acid)	10	0.087
	20	0.118
	40	0.205
	60	0.336
	80	0.401
	100	0.495
Sample(AEKKC)	100	0.356

Figure 4: Standard graph for Gallic acid for the estimation of total phenolic content



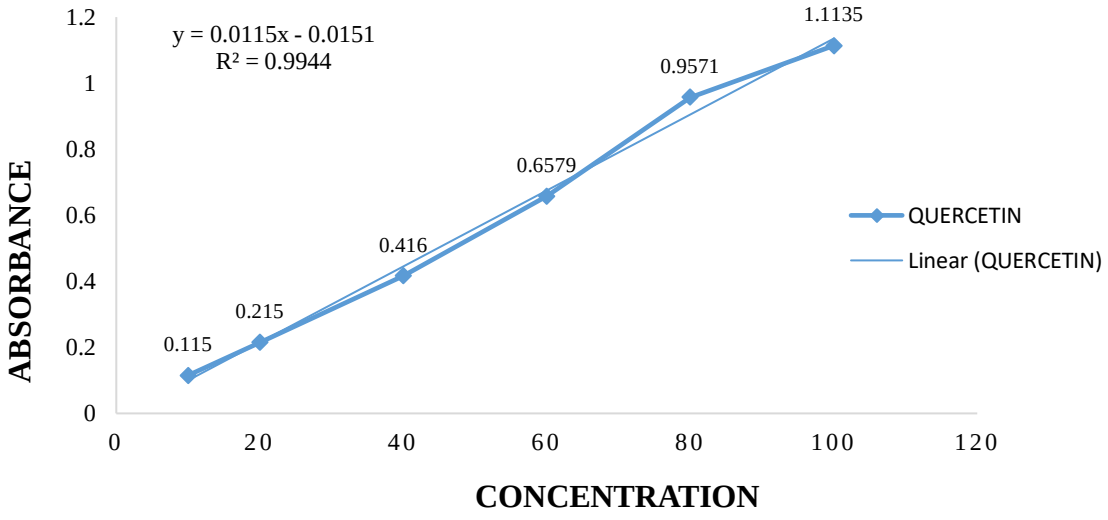
The total phenolic content in AEKKC was found to be 68.27 mg/g of extract calculated as Gallic acid equivalent.

6.2.2. ESTIMATION OF TOTAL FLAVONOID CONTENT OF AEKKC

Table 14: Estimation of total flavonoid content of AEKKC

Sample	Concentration (µg/ml)	Absorbance at 415 nm
Standard (Quercetin)	10	0.1150
	20	0.2150
	40	0.4160
	60	0.6579
	80	0.9571
	100	1.1135
Sample(AEKKC)	100	0.3643

Figure 5: Standard graph of Quercetin for the estimation of total flavonoid content



The total flavonoid content in AEKKC was found to be 32.99 mg/g of extract calculated as quercetin equivalent

6.3. ESTIMATION OF PHYTOCONSTITUENTS BY HIGH PERFOMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC study was carried out for the quantification of phytoconstituents in extract. After development the plate was scanned in densitometer under 254 nm and the chromatogram obtained is depicted in figure 7.

Figure 6: Detection of bands

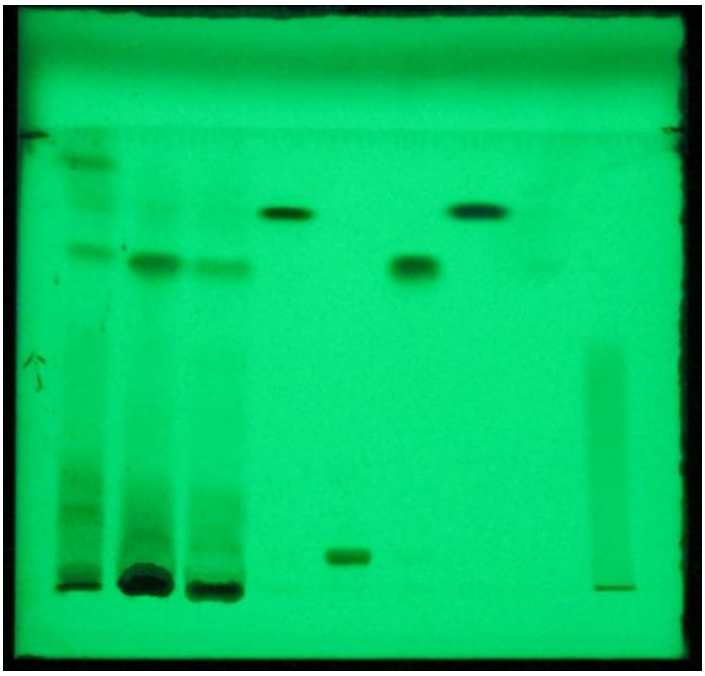


Table 15: Lists of spots applied on HPTLC plate

Track number	Sample Volume	Sample
1	10	KKC in methanol
2	10	AEKKC in water
3	10	KKC in water
4	5	Quercetin
5	5	Rutin
6	5	Gallic acid
7	5	Ferrulic acid
8	5	Andrographloide
9	5	Ellagic acid

3D DISPLAY OF CHROMATOGRAM OF STANDARDS & SAMPLES

Figure 7: 3D display of chromatogram of Samples and Standards

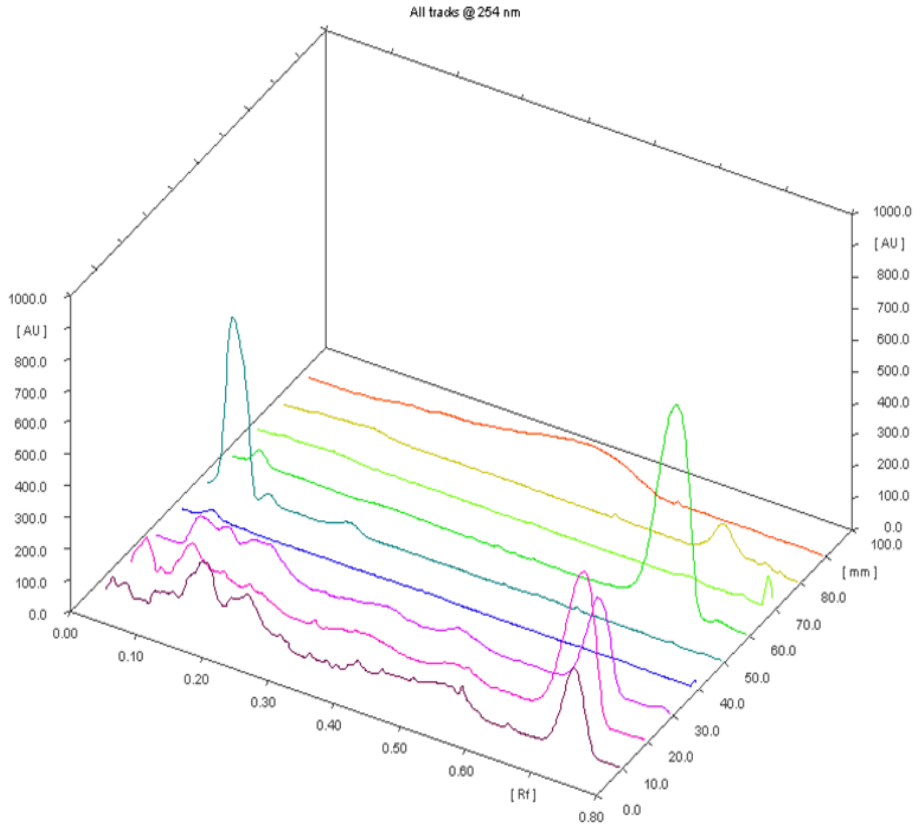
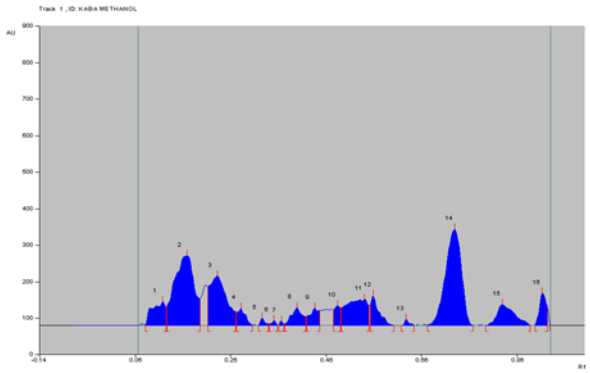
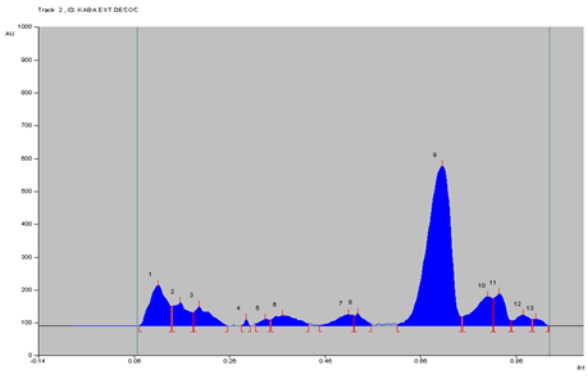


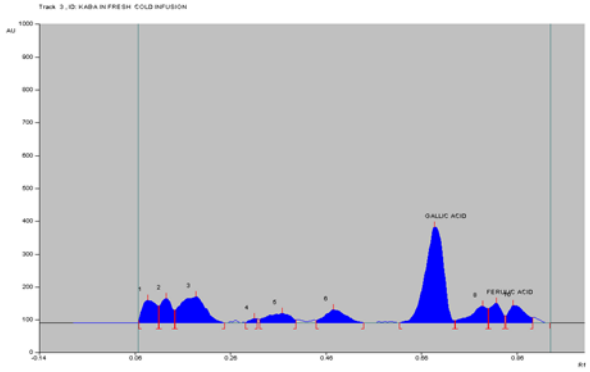
Figure 8: Chromatogram of Standards and Samples



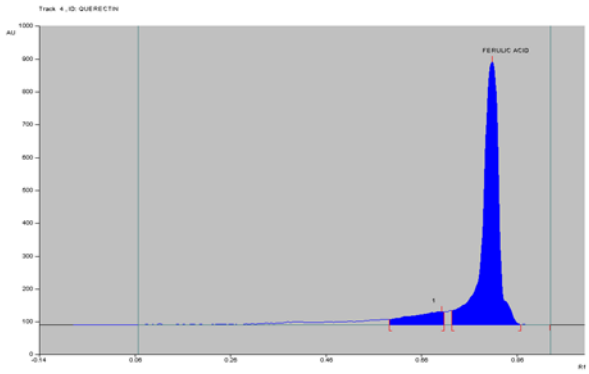
Track 1: KKC in methanol



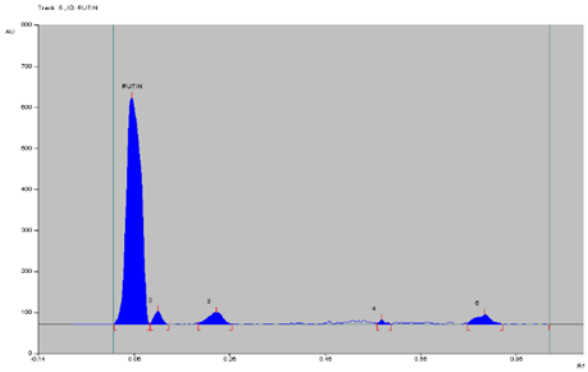
Track 2: AEKKC in water



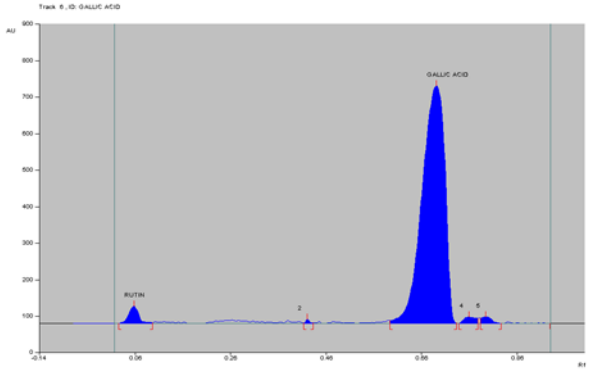
Track 3: KKC in water



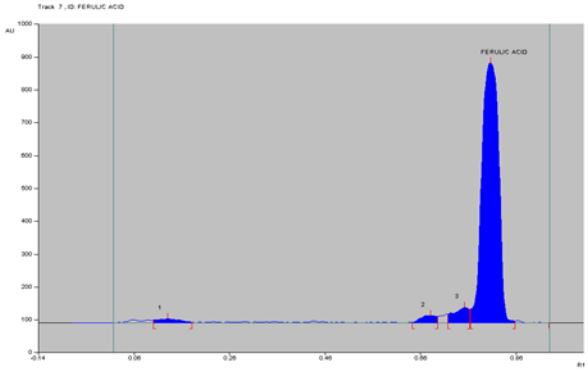
Track 4: Quercetin



Track 5: Rutin



Track 6: Gallic acid



Track 7: Ferrulic acid

6.3.1 QUANTIFICATION OF QUERCETIN AND GALLIC ACID IN EACH SAMPLES

Table 16: Amount of Quercetin & Gallic acid present in each samples

Sl. No	Track	Sample	Volume in µl	% of Quercetin in 1000 µg of extract	% of Gallic acid in 1000 µg of extract
1	Track 1	KKC in methanol	10 µl	0.03 %	0.14 %
2	Track 2	AEKKC	10 µl	0.06 %	0.37 %
3	Track 3	KKC in water	10 µl	0.02 %	0.177 %

6.4. IN VITRO ANTIOXIDANT ACTIVITY

6.4.1. DPPH RADICAL SCAVENGING ACTIVITY

Table 17: Percentage inhibition and IC_{50} values of DPPH radical by Quercetin and AEKKC

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Standard (Quercetin)	5	32.20	10.79
	10	42.14	
	15	53.36	
	20	68.33	
	25	86.65	
	30	94.56	
AEKKC	10	22.09	45.35
	20	30.94	
	40	42.53	
	60	51.21	
	80	62.22	
	100	79.23	

Figure 9: DPPH radical scavenging activity of Quercetin

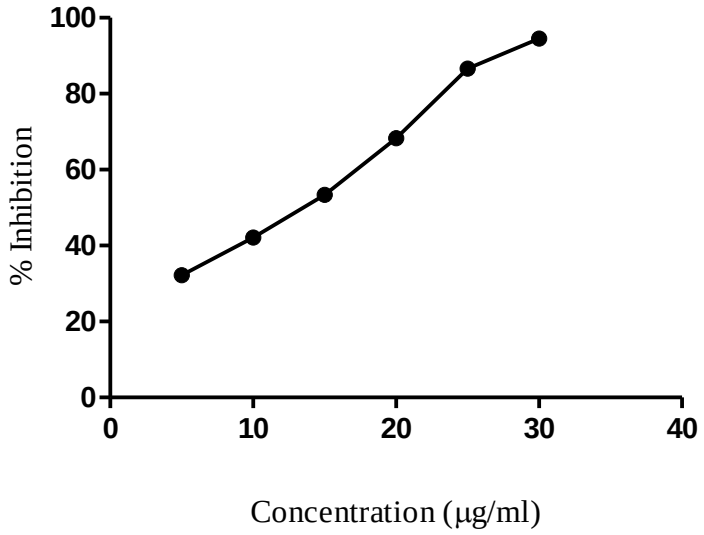
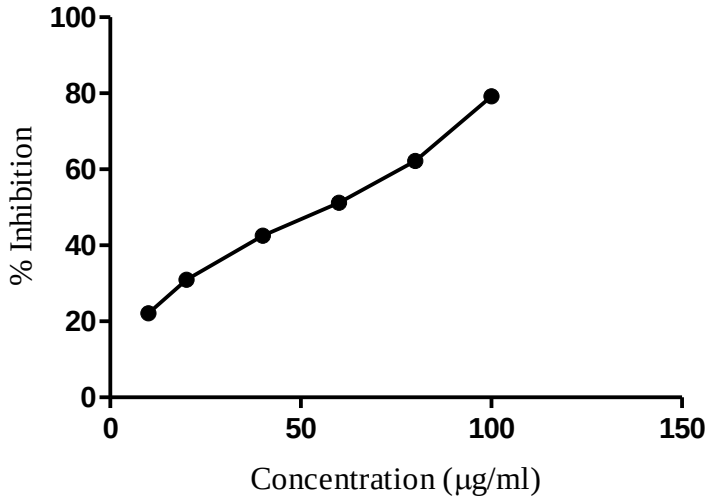


Figure 10: DPPH radical scavenging activity of AEKKC



6.4.2. ABTS RADICAL SCAVENGING ACTIVITY

Table 18: Percentage inhibition and IC_{50} values of ABTS radical by Quercetin and AEKKC

Sample	Concentration (µg/ml)	% Inhibition	IC50 (µg/ml)
Standard (Quercetin)	0.25	42.56	0.3869
	0.5	50.34	
	0.75	65.12	
	1	79.84	
	1.25	90.23	
	1.5	96.41	
AEKKC	1	25.6	3.12
	2	32.6	
	4	53.6	
	6	69.7	
	8	77.4	
	10	85.9	

Figure 11: ABTS radical scavenging activity of Quercetin

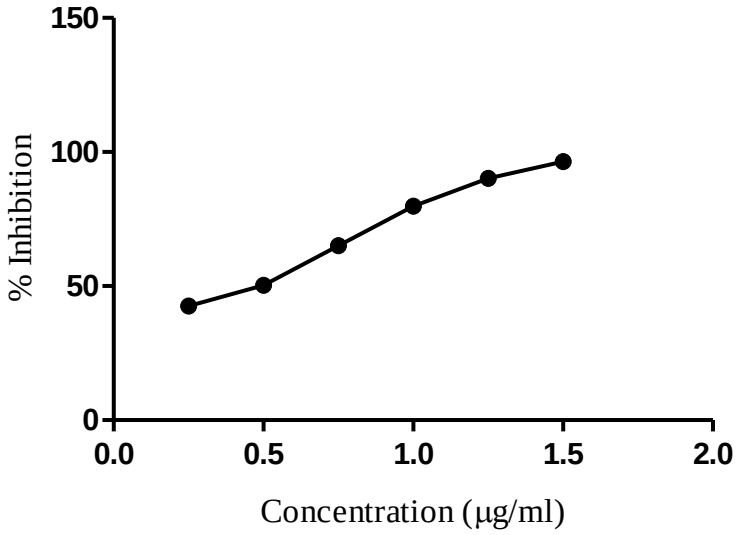
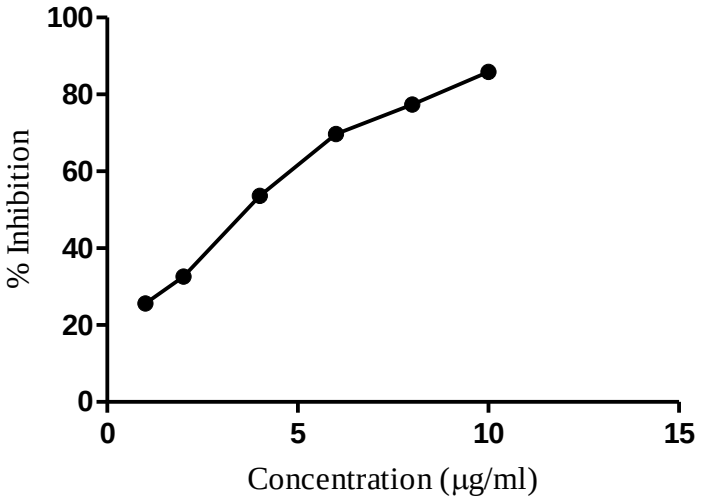


Figure 12: ABTS radical scavenging activity of AEKKC



6.5. PHARMACOLOGICAL STUDY

6.5.1. ACUTE TOXICITY TEST

The acute toxicity test was performed by using the AEKKC at concentrations of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg. Toxicity study was performed as per OECD guidelines 423. It was observed that the AEKKC was not lethal to the rats at 2000 mg/kg dose. Hence the dose fixed for study as 200 mg/kg as low dose and 400 mg/kg as high dose. The observations are summarized in table:

Table 19: Acute toxicity study

Response	Group 1	Group 2	Group 3	Group 4
Alertness	Normal	Normal	Normal	Normal
Grooming	Normal	Normal	Normal	Normal
Touch response	Normal	Normal	Normal	Normal
Torch response	Normal	Normal	Normal	Normal
Pain response	Normal	Normal	Normal	Normal
Tremor	Normal	Normal	Normal	Normal
Convulsion	Normal	Normal	Normal	Normal
Lighting reflex	Normal	Normal	Normal	Normal
Gripping strength	Normal	Normal	Normal	Normal
Pinna reflex	Normal	Normal	Normal	Normal
Corneal reflex	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Skin color	Normal	Normal	Normal	Normal
Lacrimation	Normal	Normal	Normal	Normal
Diarrhoea	Normal	Normal	Normal	Normal

6.5.2. SCREENING OF ANTI-INFLAMMATORY ACTIVITY

- **CARRAGEENAN INDUCED PAW EDEMA IN RATS**

Table 20: Effect of AEKKC on carrageenan induced paw edema in rats

Group	Treatment	Mean edema volume (ml) and % inhibition					
		0 h	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
I	Carrageenan 1%w/v (0.1ml)	0.064±0.006	0.1137±0.004	0.1870±0.005	0.2605±0.002	0.3600±0.002	0.207±0.001
II	Diclofenac (20 mg/kg)	0.067±0.003	0.090±0.008** (26.12%)	0.097±0.009*** (48.12%)	0.102±0.003*** (60.07%)	0.112±0.003*** (68.88%)	0.062±0.002*** (70.09 %)
III	AEKKC choornam (200 mg/kg)	0.067±0.004	0.102 ± 0.008^{ns} (10.02%)	0.133±0.001*** (28.87%)	0.149±0.003*** (42.69%)	0.173±0.003*** (51.72%)	0.110±0.002*** (46.93%)
IV	AEKKC choornam (400 mg/kg)	0.070±0.003	0.094±0.004** (17.32%)	0.126±0.007*** (32.62%)	0.137±0.006*** (47.30%)	0.145±0.005*** (59.72%)	0.092±0.001*** (55.33%)

Values in brackets denote percentage inhibition of the oedema paw volume

Statistical comparison: Values represent mean ± SEM, n=6 compared with control, statistical analysis was done by one way analysis of variation (ANOVA) followed by Dunnett's test. ***P<0.001, **P<0.01 and ns- non significant.

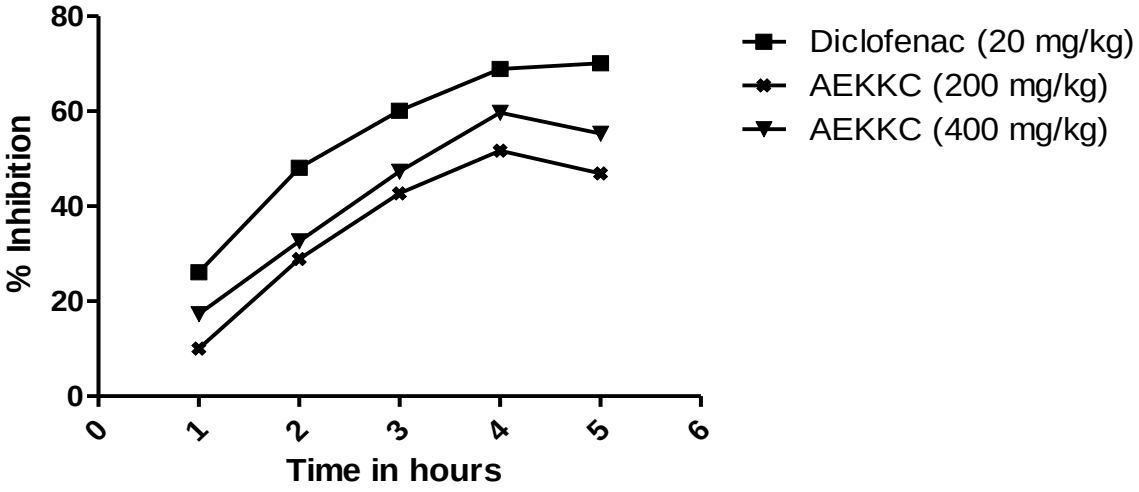


Figure 13: Anti-inflammatory effect of Diclofenac and AEKKC in carrageenan induced paw edema in rats. Each point shows percent inhibition of paw edema after 1, 2, 3, 4 and 5 h of treatment.

- **HISTAMINE INDUCED RAT PAW EDEMA METHOD**

Table 21: Effect of AEKKC on Histamine induced rat paw edema in rats

Group	Treatment and Dose	Mean edema volume(ml) and % inhibition			
		0 h	**1 h**	**2 h**	**3 h**
I	Histamine 0.1% (0.1 ml)	0.066±0.003	0.120±0.002	0.217±0.003	0.3133±0.002
II	Diclofenac (20 mg/kg)	0.067±0.004	0.061±0.0015*** (49.166%)	0.082±0.001*** (62.24%)	0.092±0.001*** (70.60%)
III	AEKKC (200 mg/kg)	0.063±0.004	0.101±0.005** (15.97%)	.112±0.001*** (48.112%)	0.130±0.002*** (58.46%)
IV	AEKKC (400 mg/kg)	0.064±0.002	0.076±0.002*** (36.83%)	0.095±0.003*** (55.89%)	0.103±0.003*** (67.09%)

Values in brackets denote percentage inhibition of the edema paw volume

Statistical comparison: Values represent mean ± SEM, n=6 compared with control, statistical analysis was done by one way analysis of variation (ANOVA) followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns- non significant.

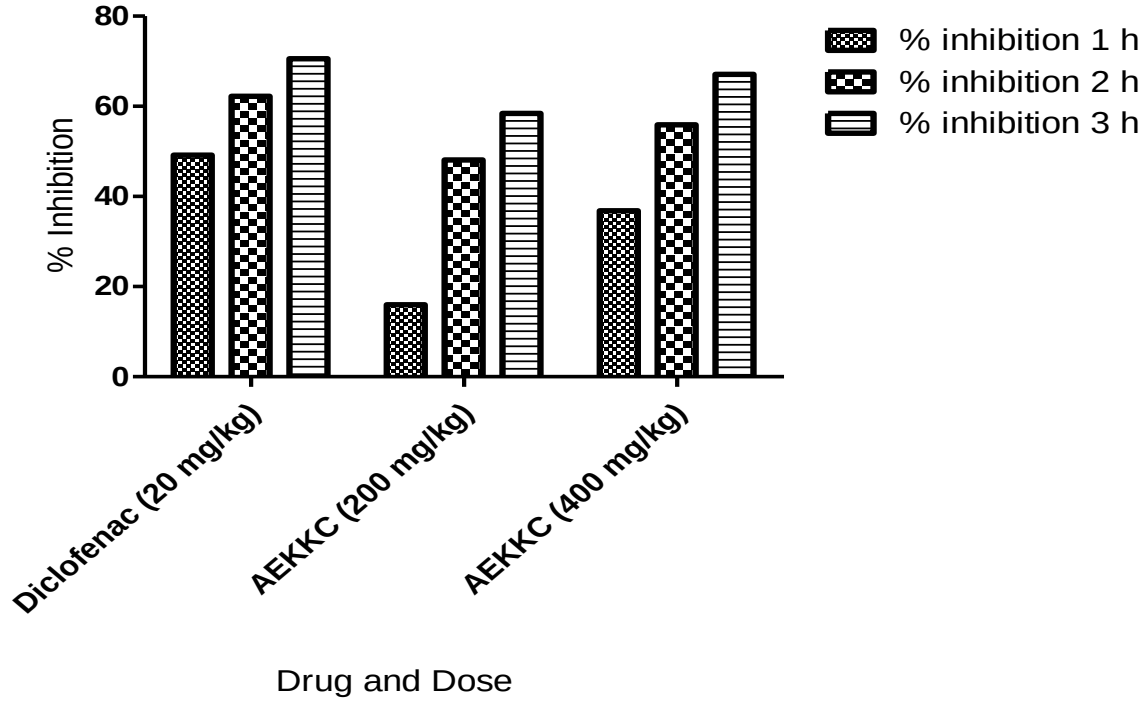


Figure 14: Anti-inflammatory effect of Diclofenac and AEKKC in histamine induced paw edema in rats.

6.5.3. SCREENING OF ANTIPYRETIC ACTIVITY

- **BREWER'S YEAST INDUCED PYREXIA MODEL**

Table 22: Effect of AEKKC on brewer's yeast induced pyrexia model in rats

Treatment	Rectal temperature in ^{o}C at various time intervals						
	-18 h	**0 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
15%brewer's yeast (10 ml/kg)	37.79±0.06	38.58±0.22	39.06±0.04	39.15±0.14	39.29±0.02	39.39±0.02	39.42±0.03
Paracetamol (150 mg/kg)	37.66±0.03	39.17±0.07	38.19±0.10*** (64.90%)	37.99±0.05*** (78.14%)	37.89±0.06*** (84.76%)	37.79±0.02*** (91.39%)	37.76±0.07*** (93.37%)
AEKKC (200 mg/kg)	37.71±0.01	39.03±0.05	38.22±0.07*** (61.36%)	38.02±0.01*** (76.51%)	37.95±0.04*** (81.81%)	37.83±0.01*** (90.90%)	37.82±0.07*** (91.66%)
AEKKC (400mg/kg)	37.71±0.09	39.09±0.09	38.70±0.06** (28.26%)	38.25±0.06*** (60.86%)	37.90±0.01*** (86.23%)	37.87±0.03*** (88.40%)	37.85±0.06*** (89.85%)

Values in brackets denote percentage reduction of rectal temperature. **Statistical comparison**: Values are expressed as Mean±SEM; n=6 compared with control, statistical analysis was done by one way analysis of variation (ANOVA) followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns- non significant

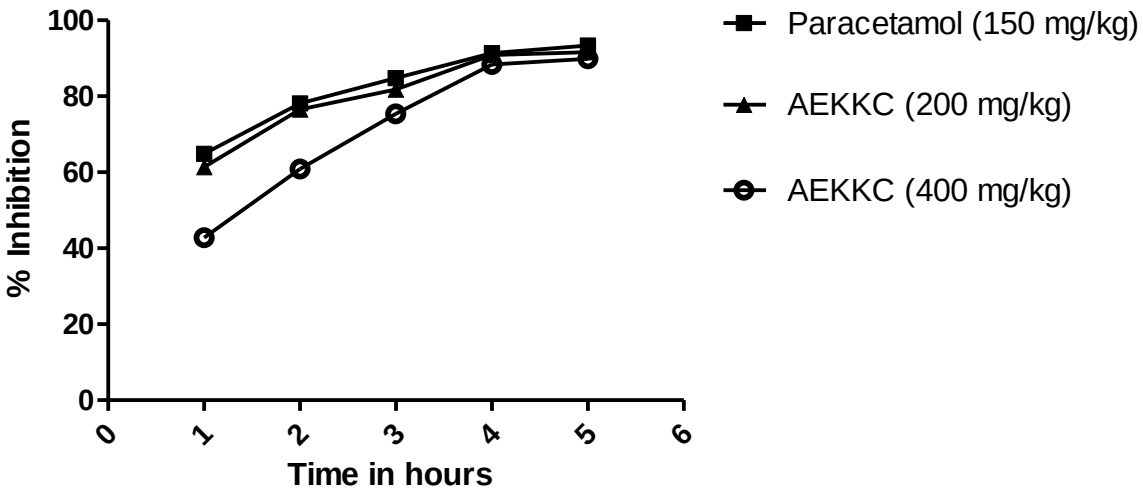


Figure 15: Anti-pyretic effect of Paracetamol and AEKKC in yeast induced pyrexia. Each point shows percentage reduction of rectal temperature after 1, 2, 3, 4 and 5 h of treatment.

6.6. ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACT OF KABASURA KUDINEER CHOORNAM

6.6.1. Zone of inhibition

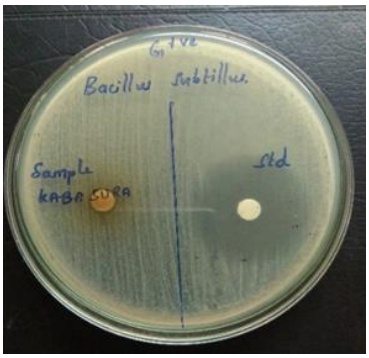
Table 23: Zone of inhibition for Gram $^{+ve}$ organisms

Organism	Standard (mm)	AEKKC (mm)
Bacillus subtilis	31 mm	10 mm
Staphylococcus aureus	35 mm	18 mm

Table 24: Zone of inhibition for Gram $^{-ve}$ organisms

Organism	Standard (mm)	AEKKC (mm)
E.coli	36 mm	11 mm
Pseudomonas aeuroginosa	38 mm	15 mm

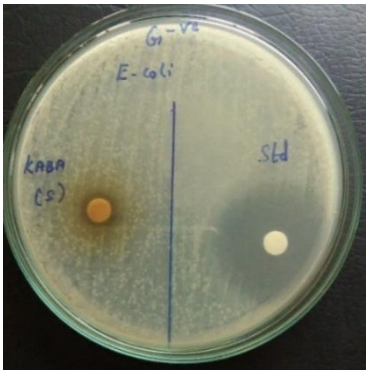
Figure 16: Zone of inhibition for Gram $^{+ve}$ organisms



Bacillus subtilis

Staphylococcus aureus

Figure 17: Zone of inhibition for Gram $^{-ve}$ organisms



E.coli

Pseudomonas aeuroginosa

6.6.2. MINIMUM INHIBITORY CONCENTRATION OF AEKKC

Table 25: MIC values of AEKKC

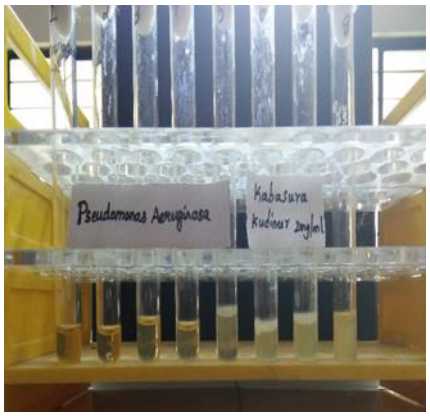
Organism	MIC value of AEKKC (µg/ml)
Bacillus subtilis	250 µg/ml
Staphylococcus aureus	250 µg/ml
E.coli	250 µg/ml
Pseudomonas aeuroginosa	250 µg/ml

Figure 18: MIC of AEKKC

Bacillus subtilis

Staphylococcus aureus

E.coli



Pseudomonas aeuroginosa

7. DISCUSSION

In the developing countries increased cost of medicine as well as their side effects has become a great task when public health is concerned. From time to time, investigations have been carried out to develop different types of polyherbal formulations to enhance the overall therapeutic potential of the formulation. And so, nowadays the traditional medical system and their herbal / herbo-mineral preparations used for various ailments are becoming more popular.

Siddha medicine is one of the oldest Indian systems of medicine. And it is most commonly practiced in India especially in southern regions. In Siddha, Kabasura kudineer choornam is widely prescribed for the management and prevention of swine flu. The phytochemical constituents present in choornam may responsible for its anti-inflammatory, antipyretic, analgesic, anti-viral, anti-bacterial, anti-fungal, anti-oxidant, hepato-protective, anti-diabetic, anti-asthmatic, anti-tussive, immunomodulatory, anti-diarrhoeal and anti-oxidant activities. [11] [97]

The current study deals with the inquiry of the effects of Kabasura kudineer choornam in two different animal models of inflammation and one animal model of hyperpyrexia.

Phytochemical screening of AEKKC was carried out and the results showed the presence of carbohydrates, glycosides, alkaloids, phenolic, tannin, flavonoids, saponins, steroids and triterpenoids.

The main attraction of the phytochemical was the presence of phenols and flavonoids which was concluded by the colorimetric estimation of these constituents in the extract. The phenolics, particularly polyphenols exhibit a wide variety of beneficial activities in mammals including antiviral, antibacterial, immune stimulating, antiallergic, antihypertensive, antiischemic, antiarrhythmic, antithrombotic, hypocholesteromic, hepatoprotective, anti-inflammatory, anticarcinogenic actions. Flavonoids are an important group of polyphenols and are reported to inhibit prostaglandin synthesis, which are known mediators of inflammation. [98]

Total phenolic content was determined by using the Folin-Ciocalteu (FC) reagent method. Plant phenols constitute the major group of compounds that act primarily as antioxidants and free radical scavengers. Hydrogen donating property of the polyphenolic compounds is responsible for the inhibition of free radical induced lipid peroxidation. [99] The total phenolic content of the AEKKC was found to be 68.27 mg/g calculated as Gallic acid equivalent.

The total flavonoid content present in the AEKKC was determined by Aluminium chloride colorimetric method. Flavonoids are natural compunds widely distributed in plant kingdom. They have great effect on mammalian enzymes like protein kinases, alpha-glucosidase and aldose reductase. Thus flavonoids regulate the altered multiple cellular signaling pathway during disease conditions. Flavonoids are categorized into 6 major subgroups based on their chemical structure such as flavones, chalcones, flavondiols, anthocyanins, flavonols and condensed tannins. [100]The total flavonoid content in extract was found to be 32.9 mg/g calculated as Quercetin equivalent.

Inorder to justify and quantify the presence phytoconstituents, the samples was subjected to HPTLC screening against marker compounds such as, Gallic acid, Quercetin, Rutin, ferrulic acid, andrographolide, ellagic acid.

From the results obtained from HPTLC study, it was found that 1000 µg of KKC in methanol, AEKKC and KKC in water contains both Quercetin & Gallic acid. KKC in methanol showed 0.03% of quercetin and 0.140% of gallic acid. While AEKKC was found to contain 0.06% of quercetin and 0.37% of gallic acid. Finally KKC in water showed 0.02% of quercetin and 0.177% of gallic acid.

Normally about 5% of the inhaled oxygen is converted to harmful ROS like O_2^-, H_2O_2, •OH. In many inflammatory disorders there is excess activation of phagocytes and production of superoxide (O_2^-) radical which can harm surrounding tissue either by a powerful direct oxidizing action or indirectly as with hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH) formed from superoxide radical. These radicals initiate lipid peroxidation and finally results in membrane destruction. The damaged tissue then provokes inflammatory response by the production of mediators and chemotactic factors. The ROS are also known to activate matrix metalloproteinase (e.g. collagenase) causing

increased destruction of tissues. Hence the agents that can scavenge these reactive oxygen species can be beneficial in the treatment of such inflammatory disorders. [98]

Antioxidant potential of AEKKC was determined by using both DPPH and ABTS scavenging method. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The free radical scavenging activity of the extract was estimated by comparing the percent inhibition of AEKKC with standard Quercetin. The IC_{50} value of extract and quercetin was found to be 45.35 and 10.79 respectively. The result showed that the extract has hydrogen donating ability and serve as free radical scavenger. ABTS assay is an excellent tool in determining the antioxidant potential of hydrogen donating antioxidants (Scavengers of lipid peroxyl radicals). From the results obtained in ABTS assay it was found that the extracts scavenged $ABTS^{+}$ radicals generated by the reaction between ABTS and potassium per sulphate. The activity was found to be increased in a dose dependent manner. IC_{50} value of Quercetin and AEKKC was found to be 0.3869 and 3.12 respectively.

Any medicine, whether synthetic or herbal is anticipated to benefit the recipient. To validate the safety and efficacy of the same, toxicity studies are of prime importance which predicts the potential toxicity of the medicine in human beings exposed to near fatal doses. The dose and extent of toxicity of the formulation was found out by acute toxicity studies according to OECD guidelines 423 in mice with the AEKKC at doses 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg. No mortality or morbidity was found till 14 days of study. The dose fixed for current study was 200 mg/kg as low dose and 400 mg/kg as high dose.

The anti-inflammatory activity of the AEKKC was evaluated by two experimental models, i.e., carrageenan-induced paw edema and histamine induced paw edema. Carrageenan induced paw edema model is used to screen the anti-inflammatory activity of a drug in the acute phase of inflammation. Edema induced by carrageenan is a biphasic event. The first phase (1 hour) involves the release of serotonin and histamine and the second phase (> 1 hour) is mediated by prostaglandins. [43] The mean edema volume and percentage inhibition was calculated for 5 hours. A significant anti-edematous activity of 200 mg/kg of AEKKC was observed during the second phase of inflammation, indicating the inhibition of prostaglandin release. While in high dose significant antiedematous was

observed from the first phase of inflammation. Both doses shows the maximal percentage inhibition at 3rd and 4th hours. The decrease in paw edema inhibition at 5th hours may be attributed to the termination of test drug action.

Both doses of extract (200 mg/kg and 400 mg/kg) exerted a significant inhibition of 15.97% and 36.83% at 1 h, 48.11% and 55.89% at 2 h, 58.46% and 69.09% at 3 h respectively in the histamine induced rat paw edema model. It was observed that the extract was capable of inhibiting edema induced by histamine and the effectiveness for suppression of edema might be due to the ability of extract to inhibit the synthesis, release or action of histamine involved in the inflammation.

Fever may be due to infection or one of the sequele of tissue damage, inflammation, graft rejection, or other disease states. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates. Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature. [101]

The present results showed that AEKKC possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug) from 1 h to 5 h. So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action of AEKKC as that of paracetamol. Also, there are several mediators or multiprocesses underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyretic effect. [101]

The anti-bacterial activity of the extract was measured by observing bacterial free zones formed around the discs. The anti-bacterial study was carried out for AEKKC against different strain of bacteria (2 Gram $^{+\ ve}$ and 2 Gram $^{-\ ve}$), that are known to cause infection in human and plants, by disc diffusion method at 200 µg/disc. The ciprofloxacin 10 µg/disc was used as standard for bacteria. The standard ciprofloxacin was found to have significant antibacterial activity against bacteria. Thus AEKKC was observed to have significant antibacterial activity.

The zone of inhibition was observed for both Gram $^{+ve}$ and Gram $^{-ve}$ bacteria. The maximum zone of inhibition (18mm) was observed for *Staphylococcus aureus* for the

Gram $^{+ve}$ organisms. And for Gram $^{-ve}$ organisms the maximum zone of inhibition (15mm) was found for *Pseudomonas aeuroginosa*.

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganism to an antimicrobial agent and it also monitors the activity of new antimicrobial agents. [102] AEKKC were found to have anti-bacterial activity with MIC of 250 µg/ml for both gram positive and gram negative organism.

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8. CONCLUSION

The present study supports that aqueous extract of **KABASURA KUDINEER CHOORNAM** (AEKKC) have significant anti-inflammatory, antipyretic and antibacterial activity.

On the basis of the study the formulation showed significant *in vitro* anti-oxidant activity by terminating the actions of free radicals. The AEKKC (200 mg/kg and 400 mg/kg) was studied for its anti-inflammatory activity using carrageenan and histamine induced inflammation. Diclofenac (20 mg/kg) was used as standard for both the models. The anti-pyretic activity was evaluated by Brewer's yeast induced pyrexia model in which paracetamol (150 mg/kg) was used as standard.

Thus the study proved that the AEKKC possesses significant ($P<0.05$) anti-inflammatory activity which was evident with reduction in mean paw edema volume in both carrageenan and histamine induced inflammatory models. In antipyretic activity, AEKKC demonstrated significant ($P<0.05$) protection by reducing yeast evoked elevated body temperature which is comparable to standard drug. Antibacterial activity was evaluated via disk diffusion method. The potential activity of extract may be due to the presence of phenols, flavonoids and other phytochemical constituents present in it.

Thus from the above results it was concluded that the AEKKC showed anti-oxidant activity and promising anti-inflammatory, antipyretic and antibacterial activity.

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